

**BIO-MONITORING FOR ATMOSPHERIC  
NITROGEN POLLUTION USING EPIPHYTIC  
LICHENS AND BRYOPHYTES**

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## **Abstract**

Nitrogen enrichment in sensitive habitats has become a matter of concern in recent years, and has led to the need to develop a bio-monitoring scheme that could be used by non-specialists to undertake site evaluation across the UK. Epiphytes are ideal candidates for such a project due to their high sensitivity to atmospheric pollutants. Indicator species analysis identified a comprehensive UK-specific list of epiphytic indicators for  $\text{NH}_3$  pollution in the UK that for the first time also included microlichens. A shortlist of easily identifiable, widely distributed indicator species was produced from these and used to trial the effectiveness of a frequency based scoring system. A simple, un-weighted frequency based scoring system founded on the Lichen Acidophyte Nitrophyte index was found to correlate strongly with  $\text{NH}_3$  concentrations in air. Assessment of other atmospheric chemistry and climate variables with the scoring system showed that  $\text{NO}_2$  concentrations in air and bark pH were confounding factors. The influence of pH was further validated by observations in a field experimentation plot where different N forms were applied. In order to address this, a regression equation was formulated that incorporated  $\text{NH}_3$ ,  $\text{NO}_2$  and bark pH to produce a predictive model that could potentially be used to evaluate site condition with respect to atmospheric N pollution, as defined by the combined effect of  $\text{NH}_3$  and  $\text{NO}_2$ . Investigations into the biochemistry of an indicator species identified that rates of phosphomonoesterase (PME) activity were higher in N-sensitive species than N-tolerant ones. N-sensitive lichen thallus pH measurements for were also consistently more acidic than those of N-tolerant species, and closer to the observed pH optima of PME activity.

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## Abbreviations (excluding standard SI units)

Abbr.	Description
AFFL <sub>AN</sub>	Accumulative and Factored Frequency-based Lichen Acidophyte Nitrophyte index
AGANet	Acid Gas and Aerosol Network
agg.	aggregation
AIW	Acidofiele Indicatie Waarde
ALPHA	Adapted Low-cost Passive High-Absorption sampler
AMFIA	Ammonium Flow Injection Analysis
AUFL <sub>AN</sub>	Accumulative, Un-factored Frequency-based Lichen Acidophyte Nitrophyte index
B% <i>fo</i>	frequency of occurrence on <i>Betula</i> spp. (%)
[base cations]	base cation ( $\text{Na}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$ ) concentration in aerosol
Bt	Bark topography
[ $\text{Ca}^{2+}$ ]	$\text{Ca}^{2+}$ concentration in aerosol
CEC	Cation Exchange Capacity
CEH	Centre for Ecology and Hydrology
CLE	Critical LEvel
DELTA	Denuder for Long-Term Atmospheric sampling
D-map	Distribution map
ECBN	Environmental Change Biodiversity Network
EI	Ellenberg Index
FL <sub>AN</sub>	Frequency-based Lichen Acidophyte Nitrophyte
FRAME	Fine Resolution Atmospheric Multi-pollutant Exchange model
gbh	girth at breast height (defined as 1.5 m from ground level)
GDD	annual Growing Degree Days > 5°C
<b>GDD</b>	mean monthly Growing Degree Days > 5°C per year
[HNO <sub>3</sub> ]	HNO <sub>3</sub> concentration in air
ISA	Indicator Species Analysis
L <sub>A</sub>	Lichen Acidophyte index
L <sub>AN</sub>	Lichen Acidophyte Nitrophyte index
L <sub>N</sub>	Lichen Nitrophyte index
[ $\text{Mg}^{2+}$ ]	$\text{Mg}^{2+}$ concentration in aerosol
[N]	total N concentration in air and aerosol
[Na <sup>+</sup> ]	$\text{Na}^+$ concentration in aerosol
NAMN	National Ammonia Monitoring Network
NBN	National Biodiversity Network
NFFL <sub>AN</sub>	Non-accumulative, Factored Frequency-based Lichen Acidophyte Nitrophyte index
[NH <sub>3</sub> ]	NH <sub>3</sub> concentration in air
[NH <sub>4</sub> <sup>+</sup> ]	NH <sub>4</sub> <sup>+</sup> concentration in aerosol
NIW	Nitrofile Indicatie Waarde
NMDS	Non-linear Multi-Dimensional Scaling
[NO <sub>2</sub> ]	NO <sub>2</sub> concentration in air
[NO <sub>3</sub> <sup>-</sup> ]	NO <sub>3</sub> <sup>-</sup> concentration in aerosol
NUFL <sub>AN</sub>	Non-accumulative, Un-factored Frequency-based Lichen Acidophyte Nitrophyte index
OI	Oceanicity Index

OPAL	OPen Air Laboratories
PDE	phosphodiesterase
PME	phosphomonoesterase
<i>p</i> NP	nitrophenol
<i>p</i> NPP	4-nitrophenol phosphate
PPMCC	Pearson's Product Moment Correlation Co-efficient
PPN	total annual precipitation ( $\text{mm y}^{-1}$ )
Q:B	<i>Quercus</i> : <i>Betula</i> ratio of frequency of occurrence (%)
Q% <i>fo</i>	frequency of occurrence on <i>Quercus</i> spp. (%)
RD	Rain Days
[S]	total S concentration in air and aerosol
s.l.	<i>sensu lato</i>
[SO <sub>2</sub> ]	SO <sub>2</sub> concentration in air
SO <sub>4</sub> <sup>2-</sup> <sub>NSS</sub>	non-sea salt SO <sub>4</sub> <sup>2-</sup>
[SO <sub>4</sub> <sup>2-</sup> ] <sub>NSS</sub>	non-sea salt SO <sub>4</sub> <sup>2-</sup> concentration in aerosol
sp.	species (singular)
spp.	species (plural)
SR	species richness
SRC	Spearman's Rank Correlation
s.s.	<i>sensu stricto</i>
T	mean annual temperature (°C)
T <sub>max</sub>	mean annual maximum temperature (°C)
<b>T</b> <sub>max</sub>	mean annual monthly maximum temperature (°C)
T <sub>min</sub>	mean annual minimum temperature (°C)
<b>T</b> <sub>min</sub>	mean annual monthly minimum temperature (°C)
UK	United Kingdom
UNECE	United Nations Economic Commission for Europe

## **Chapter 1. Introduction**

### **1.1 Background**

Many natural plant communities in the UK have developed as a result of low N input (Grime, 2001; Leith *et al.*, 1999), and include habitats such as acid grassland, calcareous grassland, Caledonian pine forest, low-alpine heath, moorland, ombotrophic bog and sand dune systems. Anthropogenic emissions of reactive N have increased dramatically since the industrial revolution (Holland *et al.*, 1999). Consequently, the threat of N pollution as a principal driver of biodiversity loss in naturally nutrient-poor ecosystems has become a matter of concern to the point it has been defined as the main threat to northern hemisphere temperate habitats. Predictions by Sala *et al.* (2000) of future global biodiversity change ranked N-deposition as the third most influential driver, next to land use and climate change. Furthermore, the influence of N-deposition is estimated to be three times greater than any other driver in northern hemisphere temperate habitats (Sala *et al.*, 2000).

Rural N emissions to the atmosphere are typically in the reduced state as  $\text{NH}_3$ , and predominately originate from agricultural practices including: cattle, poultry sheds, pig pens and fertiliser application. Clear negative relationships have been shown between N deposition and overall species richness (SR) in both acid grassland and prairie vegetation, whilst biomass and cover of a few species increase (Stevens *et al.*, 2004; Stevens & Tilman, 2010). A typical pattern exists whereby total SR and cover of forbs and lichens decline, whilst graminoid cover increases (Gordon *et al.*, 2001; van der Eerden *et al.*, 1998).

This trend of a shift toward the dominance of a certain type of graminoid species at the cost of other species has been shown in numerous studies across various habitat types including: *Festuca ovina* in alpine communities, *Brachypodium pinnatum* in chalk grassland *Nardus stricta* in heathland and *Molinea caerulea* in ombrotrophic bog (Bobbink & Willems, 1987; Fremstad *et al.*, 2005; Leith *et al.*, 1999; Tomassen *et al.*, 2004).

The dominance of a few productive species to the overall detriment of total SR has been described as being the result of inter-specific competition (Leith *et al.*, 1999; Tilman, 1997). However, the deleterious effect of N-enrichment is not solely restricted to increased competitive interactions. High N deposition is linked to increases in soil acidity and the subsequent leaching of base cations and greater availability of phytotoxic metals (Bobbink *et al.*, 1998; Högberg *et al.*, 2006). It has also been shown that the impacts of secondary stress factors, e.g. drought, frost, oxidative stress and pathogens, are greatly increased under high N conditions (Bobbink *et al.*, 1998; Cross *et al.*, 1998; Flückiger & Braun, 1998; Leith *et al.*, 2001).

Because of the threat of N driven changes in community composition, there is a need to monitor N levels to identify potential threats to sensitive habitats, especially in areas where detrimental land management changes arise. Numerous physicochemical methods of measuring different N forms exist, both as concentration in air and wet and dry deposition. However, the investment required for these monitoring approaches in terms of cost and man hours is much greater than a simple biological method field assessment. A

straightforward bio-monitoring scheme for identifying deleterious N effects would therefore prove a beneficial means of initial evaluation of site condition with regards to N pollution. As  $\text{NH}_3$  is the principal source of atmospheric N in rural locations, the primary requisite for any bio-monitoring scheme needs to consider the use of indicator species for atmospheric  $\text{NH}_3$ . Furthermore, the species used in the bio-monitoring scheme require a high level of sensitivity relative to the habitats surveyed, such that the changes in indicator species composition are identifiable before total site damage becomes too extensive.

Epiphytic lichens are good candidates for atmospheric  $\text{NH}_3$  indicator species due to their lack of a root system and subsequent reliance on airborne nutrients such as N and P. Furthermore, evidence exists to suggest that epiphytic lichen community structure is linked to N supply (Fрати *et al.*, 2007; Ruoss, 1999; van Dobben & de Bakker, 1996; van Herk, 1999). Many of the epiphytic lichen communities found in the UK are typical of acidic and/or nutrient-poor environments, and very few associated with nutrient enrichment (James *et al.*, 1977). This fact, combined with the knowledge that epiphytes are typically more sensitive to atmospheric pollution than terricolous plants means they are potentially better indicators of atmospheric N pollution in sensitive habitats.

Research was recently undertaken investigating the use of epiphytes in a UK-wide bio-monitoring scheme for  $\text{NH}_3$  (Leith *et al.*, 2005<sup>a</sup>; Wolseley *et al.*, 2005<sup>a</sup>). Wolseley *et al.* (2004) and Wolseley *et al.* (2005<sup>a</sup>) undertook assessments of three European epiphytic bio-monitoring techniques and identified the Lichen Acidophyte/Lichen Nitrophyte ( $L_A/L_N$ ) method as a

potentially easy and rapid means of effectively assessing  $\text{NH}_3$  pollution. Wolseley *et al.* (2009) then revised the  $L_A/L_N$  system to produce the Lichen Acidophyte Nitrophyte ( $L_{AN}$ ) method. This method utilises the relative frequencies of lichen species tolerant to either acidic (acidophytic) or eutrophic (nitrophytic) conditions, as defined by van Herk (1999). One problem with this assessment was that it focussed primarily on the epiphytic macrolichens in van Herk's list, giving marginal consideration to other epiphytes. In addition, it utilised an indicator species list that was based on research undertaken in the Netherlands (van Herk, 1999; van Herk *et al.*, 2002), and hence, not specific to the UK. However, the work did highlight the importance of several other factors aside from increasing  $[\text{NH}_3]$  that determine lichen community composition in the UK. Most notable of these were changes in bark pH and mean annual precipitation (Wolseley *et al.*, 2005<sup>a</sup>). In particular, because of co-variance between substratum pH and  $[\text{NH}_3]$ , the relative importance of these two factors was difficult to quantify.

With these issues in mind, this project aimed to expand upon this work by identifying a UK specific list of lichen indicator species for  $\text{NH}_3$  pollution, which for the first time considered both macro- and microlichens, and to assess the influence of other key environmental variables (e.g. precipitation) on epiphytic community structure. With this information a quick and simple bio-monitoring scoring system, based upon the  $L_{AN}$  method, was formulated for use in site assessments by regulatory and conservation agency staff in the UK. The following is a review of the subject matter and highlights some of the problems that needed to be considered.



## **1.2 Nitrogen**

### **1.2.1 The Various Forms and Effects of Atmospheric N**

Nitrogen is one of the main plant macro-nutrients that, when present in large quantities, deleteriously alters the floristic composition of natural and semi-natural communities by reducing SR (Silvertown *et al.*, 2006). Numerous examples of the influence of N-enrichment have been recorded, including its effect on pine forest bryophytes (Dirske & Martakis, 1999), upland heathland (Leith *et al.*, 1999), chalk grasslands (Bobbink & Willems, 1987) and lichen-rich alpine communities (Fremstad *et al.*, 2005). It is an atypical plant nutrient in the sense that its major pool is atmospheric (in the form of  $N_2$ ) and not lithospheric. N-fixing plant symbioses (e.g. legumes, cycads and lichens) can access the large atmospheric pool of  $N_2$  directly through their symbiotic relationship with diazotrophic bacteria or cyanobacteria. Some plants and most micro-organisms also utilise organic N, which can comprise up to 50% of total N deposition in some parts of the UK (Chapman *et al.*, 2011). The bioavailability of this N source has been described as being largely unknown (Benítez, 2010), although uptake in the forms of urea (Vicente *et al.*, 1984) and the amino acid glutamine (Palmqvist & Dahlman, 2006) by lichens has been proven. Given the general uncertainty, and the numerous complex relationships between different N species, only inorganic N forms have been considered in this research.

The mobile inorganic N forms  $NH_4^+$  and  $NO_3^-$  are more readily available, and acquired by almost all plants. The importance of  $NO_3^-$  and  $NH_4^+$  for plant growth has been exploited by humans for many years through the application

of fertilisers in various forms, including  $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_3$ . Although a boon for agriculture, there have been deleterious effects of excessive or inappropriate fertiliser application. For example, eutrophication of water courses and terrestrial habitats has been identified as a cause of concern as a driver of habitat degradation and species loss (Lucassen *et al.*, 2003; Willems, 2001). The negative consequences of excessive concentrations of mobile N forms are not restricted to soils and water courses. Elevated atmospheric  $\text{NH}_3$  concentrations originating from livestock also has adverse effects through eutrophication and acidification. The impact of atmospheric N has been observed to be particularly great in naturally nutrient-poor habitats and on epiphytic species, with high reactive N levels being directly linked to changes in species composition (Frati *et al.*, 2007; Kricke & Feige, 2004; Wolseley *et al.*, 2006<sup>a</sup>; van Herk *et al.*, 2003<sup>a</sup>; van Herk *et al.*, 2003<sup>b</sup>; van Dobben *et al.*, 2001; van Herk, 1999; Sjøchting, 1995; de Bakker, 1989).

The different forms of N pollution can be broadly segregated into two types: oxidised and reduced. This is a convenient means of separation as the main sources for these types also segregate in broad geographical terms. The sources of oxidised N (e.g.  $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{HNO}_3$ ) are primarily located in and around urban environments, and produced as by-products of the combustion processes. High level emissions of oxidised N, e.g. from power stations, constitute a key component of long-distance pollution, with the majority of deposition as  $\text{NO}_3^-$  occurring at distances > 1000 km from source point (NEGTA, 2001). Low level  $\text{NO}_x$  sources, e.g. vehicular emissions, disperse over much shorter distances; for example, Cape *et al.* (2004) showed

that [NO<sub>2</sub>] values are greatest adjacent to major roads, and that concentrations decline by 90% over a distance of 15 m. Oxidised N forms principally impact upon epiphytic plant community composition through short-distance dispersion and deposition. This is particularly evident in urban environments where exposure to vehicular emissions is very heavy (Larsen *et al.*, 2007; Purvis *et al.*, 2003), although the influence of other urban environmental factors (e.g. alkaline dust from roads and buildings etc.) may compound NO<sub>x</sub> effects (Fuller & Green, 2004; Loppi *et al.*, 1997; Gilbert, 1976).

Conversely, reduced N (NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>) is predominantly rural in origin, much being derived from livestock excreta. Thus it is principally emitted from low level sources, with the majority being deposited at distances < 1000 km from point source (NEG-TAP, 2001). The effects of NH<sub>3</sub> on plants and the environment are complex. Dry deposition of NH<sub>3</sub> only occurs over short distances from the emission source, due to the low density and rapid dispersal and reduction of the gas. As in the case of NO<sub>2</sub>, atmospheric [NH<sub>3</sub>] along roadside transects in Scotland have been found to fall rapidly, by 90% within the first 10 m (Cape *et al.*, 2004). Data from transects along an [NH<sub>3</sub>] gradient through a woodland show how the deposition of NH<sub>3</sub> decreases steadily to near background levels over a distance of ca. 300 m (Fowler *et al.*, 1998). Conversely, the wet deposition of NH<sub>4</sub><sup>+</sup> acts as a means of long distance dispersal of reduced N, depositing it at distances of up to 1000 km (Asman & van Jaarsveld, 1990).

### 1.2.2 N and Epiphytic Communities

Research investigating the impacts that atmospheric N has on lichens in rural and urban environments has focussed primarily upon the effects of  $\text{NH}_3$  and  $\text{NO}_2$  concentrations respectively (Davies *et al.*, 2007; Frati *et al.*, 2007; Wolseley *et al.*, 2006<sup>a</sup>; Wolseley *et al.*, 2006<sup>b</sup>; Batty *et al.*, 2003; van Herk, 1999; Wolseley & Pryor, 1999; van Dobben & de Bakker, 1996). However, van Herk *et al.* (2003<sup>a,b</sup>) argue that the potential added effects of other forms of N, such as wet deposited  $\text{NH}_4^+$  and  $\text{NO}_3^-$  also need consideration. If  $\text{NH}_4^+$  and  $\text{NO}_3^-$  deposition can also modify lichen communities then the effects on lichens of ionic N across Europe might have some parallels with the past impacts of S pollution.

The findings of a species cover survey on a 10-year fertilisation experiment site by Fremstad *et al.* (2005) in oligotrophic Norwegian lichen-rich alpine communities do not support the hypothesis that long distance wet deposited N pollution affects epiphytic communities. They suggest that climate, soil properties and community structure are more important. This was a study of terricolous lichens and not directly comparable with the epiphytic work of van Herk *et al.* (2003<sup>a,b</sup>). However, it highlights a critical issue for any investigation into the effects of atmospheric N on epiphytes, which is that other environmental factors must be considered. The composition of epiphytic lichen communities, like those of terricolous lichens, has been linked to field-layer vegetation; both of these factors, in turn, have been linked to soil properties (Gustafsson & Eriksson, 1995; Gauslaa, 1985). This is likely due to the combined processes of stemflow, throughfall (Hauck *et al.*, 2002) and dust

impregnation (Loppi & Pirintsos, 2000). The community structure of epiphytes, together with microhabitat variation, also play key roles in processes such as competition and facilitation, and these are influential in species composition. These factors must be considered when attempting to identify species as indicators for a bio-monitoring scheme. The most influential factors that require consideration are discussed in greater depth below.

Several landscape-scale studies have been undertaken to investigate the impacts of  $\text{NH}_3$  on epiphytic lichens and have involved transects away from a point source (Frati *et al.*, 2007; Wolseley *et al.*, 2006<sup>a</sup>; van Dobben *et al.*, 2001; Sjøchting, 1995; de Bakker, 1989). In each case the results show a preference by some species (the nitrophytes) for areas of high  $[\text{NH}_3]$ , and others (the acidophytes) for areas of low  $[\text{NH}_3]$ ; furthermore, the same species re-occur amongst the nitrophytes and acidophytes in each study. In addition, bark pH measurements were also taken and were consistently found to be positively correlated to increasing  $[\text{NH}_3]$  (Frati *et al.*, 2007). While such correlations have been demonstrated at the landscape scale in the UK in relation to point sources of  $\text{NH}_3$  (Wolseley *et al.*, 2005<sup>b</sup>), they were not evident at the regional (nationwide) scale in response to regional variation (Wolseley *et al.*, 2005<sup>a</sup>). Identifying the specific importance of the effects of increasing pH and  $[\text{NH}_3]$  on lichen communities is challenging, but van Herk (2001) has previously proposed that bark pH is more important, and that the influence of  $\text{NH}_3$  is primarily that of a driver in pH change rather than that of direct physiological effects on lichens *per se*.

### 1.2.3 N and Bark pH

In a study of two transects in the Netherlands running from a nature reserve to an agricultural area, de Bakker, (1989) concluded that high bark pH, resulting from the effects of high  $[\text{NH}_3]$ , biased lichen communities in favour of nitrophytes. It was also noted by de Bakker that bark pH was more important than N supply based on two observations. The first by Gilbert (1976) was that nitrophytes were found in areas with high levels of calcareous dust but where there was no evidence of N pollution. Gilbert's interpretation was that bark pH was elevated as a result of the calcareous dust, which promoted nitrification, and consequently eutrophication; and thus, was responsible for the indirect development of the nitrophytic *Xanthorion* community. The second observation was that nitrophyte abundance correlated with bark pH, but not with bark  $\text{NH}_4^+$  concentrations, even though the community composition as a whole was influenced by  $\text{NH}_4^+$  after corrections for pH were made. This statement suggests although bark pH may be an important factor for non-nitrophytic lichens, availability of  $\text{NH}_4^+$  in bark might also have an effect on them.

Work in the Netherlands by van Dobben *et al.* (2001) found that the above environmental variables were ranked in decreasing influence on epiphytic lichens as follows: bark pH > bark  $[\text{NH}_4^+]$  >  $[\text{NH}_3]$ . These results thus corroborate those of de Bakker (1989). It was also shown by van Dobben *et al.* (2001) that  $\text{SO}_2$  and  $\text{NO}_2$  had the greatest impact on epiphytic lichens of all the factors assessed in the study. This supports the suggestion by van Herk *et al.* (2003<sup>a,b</sup>) that other N forms need to be considered. It also provides a warning

not to overlook the potential effects of other pollutants completely, including SO<sub>2</sub> (see also Section 1.4.1.4).

Understanding the relative importance of NH<sub>3</sub> and bark pH as determinants of epiphytic community composition is fundamental for classifying potential indicator species. Although the terms acidophyte and nitrophyte are widely used, the very use of these two terms alludes to the uncertainty of using the current species grouping. If the bio-monitoring is directed towards monitoring atmospheric N pollution, then the indicator species need to be selected based upon the effect that N enrichment has upon them (i.e. nitrophobes and nitrophiles), and not based upon a group of species that have a preference to either acidic (i.e. acidophytes) or N-enriched conditions (i.e. nitrophytes). This is an important point given that different N forms have different effects on substratum pH (Sutton *et al.*, 2009; ap Simon *et al.*, 1987; van Breeman *et al.*, 1982). This issue is less important if the goal is to monitor solely for NH<sub>3</sub>, given the positive correlation between [NH<sub>3</sub>] and bark pH, as indicator species identified as NH<sub>3</sub>-based nitrophobes are also likely to be acidophytic.

Whether indicator species are described as acidophytes/nitrophytes or nitrophobes/nitrophiles might be considered academic. However, it raises two important matters. First, how are epiphytes being affected by high N levels, both in air and precipitation; and, what is causing the loss and/or deterioration of sensitive species (i.e. is it a result of N, bark pH, or a combination of both)? The second issue reiterates the importance of addressing the influence of potentially confounding factors as highlighted by Krupa (2003), which can be

considered either before establishing the methodologies of any field investigation (in an attempt to limit their influence), or during the analysis (to identify bio-monitoring indicator species). Potential confounding factors might be either biotic or abiotic, and are discussed later (Sections 1.5 and 1.6).

### **1.3 Epiphytes as Bio-monitors**

An epiphyte is defined as a plant that grows on another plant. They lack soil penetrating roots and live above the ground surface on a supporting structure (Allanby, 1998; Bailey, 2006). Epiphytes obtain nutrients from the air, rainwater and organic debris on their supporting structure. Nutrient deposition in solution can occur either by rainfall (snowfall) or occult precipitation (see Sections 1.4.1.1). Rainfall can be either intercepted directly by epiphytes, or acquired indirectly via the phorophyte as throughfall and stemflow.

Epiphytes have been used previously in bio-assessments, a classic example being their application as bio-monitors for estimating [SO<sub>2</sub>] pollution (Hawksworth & Rose, 1970). Epiphytic lichens and bryophytes are good plant bio-monitors of atmospheric pollution for three key reasons:

1. They are more sensitive to atmospheric changes than other plants, as they absorb mineral ions readily across their whole surface area due to their poikilohydric nature and virtual lack of cutin (Pitcairn *et al.*, 1995). A recent publication proposed that a long-term NH<sub>3</sub> critical level (CLE) of 1 µg m<sup>-3</sup> for bryophytes and lichens be adopted by the United Nations Economic Commission for Europe (UNECE) (Cape *et*



*al.*, 2009). This value is lower than the CLE value of  $3 \mu\text{g m}^{-3}$  set by the UNECE for vascular plants (Cape *et al.*, 2009), reflecting their greater sensitivity to contaminated atmospheres.

2. Epiphytes do not directly access nutrients in the soil. The only acquisition of soil-based material occurs *via* the processes of phorophyte stemflow, throughfall, or windblown dust.
3. Epiphytic lichens do not have a seasonal life-cycle, meaning they can be sampled for surveying throughout the year.

It is possible that not all of the species comprising the observed epiphytic community in a given location will react in the same way to increased N levels. Consequently, specific indicator species need to be identified from within the whole community, and it is therefore evident that the detail of any bio-monitoring scheme is most effective at the species level. Three bio-monitoring schemes that have been established at the species level in Europe for indicating N-enrichment are the Ellenberg Index (Ellenberg *et al.*, 1992; Ellenberg 1979), the Acidofiele Indicatie Waarde Indices/Nitrofiele Indicatie Waarde Indices (van Herk, 1999) and the Lichen Acidophyte Nitrophyte Index (Wolseley *et al.*, 2009).

### **1.3.1 Ellenberg Index**

The Ellenberg Index (EI) (Ellenberg, 1979) scores species based upon their resilience/preference to a number of variables, but only considered vascular

plants in its original conception. Subsequently, lists with scores for lichens in mainland Europe (Wirth, 1991), and bryophytes in the UK and Ireland (Hill *et al.*, 2007) were produced. However, no UK lichen list that factors into it the more oceanic climatic influence exists for use in the Ellenberg system. Consideration was given to the formulation of a detailed UK-specific EI scale for epiphytic lichens, but concerns about its practicality were raised in a study by Wolseley *et al.* (2005<sup>b</sup>) who showed that, although reliable results were obtained in relation to N-tolerant lichen species, the EI was not suitable for assessing N-sensitive species loss. The EI was not deemed practical for two further reasons.

First, the EI involves a full botanical survey and, therefore, good skills in field identification. This is inconsistent with the main purpose of the bio-monitoring scheme, which is to provide a monitoring tool to Government conservation agency staff with limited knowledge of lichen and bryophyte identification. Furthermore, comprehensive species surveys are time consuming. Therefore, an approach that uses a smaller sub-set of easily identifiable indicator species would be more appropriate.

Second, although very detailed in its coverage of influential ecological factors, the EI method, as can be observed in the assessment for British and Irish bryophytes (Hill *et al.*, 2007), does not differentiate between N forms. The EI classifies total N deposition as a general indicator of fertility. Accordingly, it neither assesses [NH<sub>3</sub>] as an individual factor, nor does it consider the possibility that certain N forms may have different effects on epiphytes. This

may be particularly important in the case of lichens given the potential importance of bark pH in addition to different N forms in their ecology (see Sections 1.2.3 and 1.4.2.2).

### **1.3.2            Acidofiele Indicatie Waarde and Nitrofile Indicatie Waarde Indices**

The Acidofiele Indicatie Waarde (AIW) and the Nitrofile Indicatie Waarde (NIW) indices formulated by van Herk (1999) provide very effective methods for quantifying N pollution based on lichen community composition. They are defined by two separate indicator species lists: one of acidophytes (species that favour low pH and low  $[\text{NH}_3]$ ) and one of nitrophytes (species favouring high pH and high  $[\text{NH}_3]$ ). These lists are comprehensive, including both macro- and microlichens, and have been used effectively in the Netherlands (van Herk, 1999). The practical application of the AIW/NIW Indices in UK bio-monitoring is unfortunately limited because of two aspects of the species lists. First, both the acidophyte and nitrophyte lists were established for use in continental Europe, and specifically the Netherlands, in a region and at a time when  $[\text{NH}_3]$  values were considerably higher than those currently found in the UK (NAMN, 2009; van Herk, 1999). Therefore, as a result of spatial differences relating to atmospheric pollutant concentrations and species response curves, van Herk's lists would potentially omit species regarded as nitrophytes in a UK bio-monitoring scheme. Second, N-sensitive epiphytic communities in the UK contain species that are not present in the Netherlands either because they have an oceanic range, or because they were already eliminated in the Netherlands by pollution before the lists were constructed.

Hence, the use of these exact lists in the UK, which could potentially exclude key acidophytic species, would result in erroneously low AIW values.

In addition the AIW/NIW indices do not accommodate the environmental gradients found across the UK. These include both the longitudinal precipitation gradient, which divides the UK into the oceanic west and more continental east, and the latitudinal temperature gradient. Corresponding east-west geographical gradients exist in the UK distributions of acidophytic lichens, which have been linked to the influences of rainfall, altitude and occult precipitation (Wolseley & James, 2002; Wolseley *et al.*, 2005<sup>a</sup>).

### **1.3.3 $L_A/L_N$ and $L_{AN}$ Indices**

The Lichen Acidophyte ( $L_A$ ) and Lichen Nitrophyte ( $L_N$ ) Indices were adopted by Wolseley *et al.* (2005<sup>a</sup>) to produce a simplified version of the AIW/NIW method that they trialled in the UK, utilising a shortlist of macrolichens based on the AIW/NIW system (Wolseley, 2005). One aim of the  $L_A/L_N$  system was to produce a practical means of assessment that could be used by non-specialists. Consequently, the  $L_A/L_N$  would be a far more efficient method than the EI or AIW/NIW, requiring shorter survey times and a reduced need for training and skill levels amongst surveyors. This latter point is of particular importance given the difficulty of identifying many epiphytic cryptogams.

Sutton *et al.* (2009) subsequently adapted the  $L_A/L_N$  approach by combining the  $L_A$  and  $L_N$  scores to form the  $L_{AN}$  index, which utilised the most easily identifiable macrolichen species in the field. At the end of a series of field

studies, it was found that this new  $L_{AN}$  scoring system correlated well with site  $[NH_3]$  values (Sutton *et al.*, 2009). However, although the indicator species used in this work were a more refined selection of macrolichens, an objective assessment of potential indicators for the UK as a whole had still not been undertaken at this stage. Sutton *et al.*'s (2009) indicator species excluded microlichens, which not only constitute a large proportion of UK lichen diversity, but could be of particular importance in lichen communities on branches which include earlier successional stages in epiphytic lichen community development.

#### **1.4 Potentially Confounding Variables**

Phytosociological classifications of lichen communities have been proposed based upon observed patterns in species distributions, which are influenced by numerous variables (Barkman, 1958<sup>a</sup>; Barkman *et al.*, 1976; Frenkel & Harrison, 1974; Hale, 1955; James *et al.*, 1977; Shimwell, 1971). Therefore in addition to  $[NH_3]$ , the influence of other environmental and ecological factors on epiphytic species composition requires consideration. N pollution is the most noteworthy factor linking cryptogamic phytosociology with the impacts on the *Xanthorion parietinae* Alliance (in particular the *Xanthorion parietinae* and *Physcietum caesia* associations), which contain many species listed as nitrophytes (James *et al.*, 1977; van Herk, 2002; Wolseley, 2005).

These two phytosociological associations are described for saxicolous and corticolous communities, occurring both naturally and as a result of nutrient enrichment. They are distributed widely throughout the British Isles, especially

in the drier south and eastern lowlands (James *et al.*, 1977). It should also be noted that while particularly frequent on limestone, the associations are less specific to rock type than nutrient availability. Their catholic distribution across different substrata means that the application of a bio-monitoring scheme can be considered in principle for corticolous, lignicolous and saxicolous species. However, a level of selectivity is required to eliminate bias resulting from naturally occurring associations, particularly when attempting to identify potential indicator species. Therefore potential confounding factors had to be considered and either selectively excluded from, or factored in to, the analyses. All the potentially confounding variables can be broadly classified as being either abiotic or biotic in origins, and consideration was given to the following.

#### **1.4.1 Abiotic Factors**

##### **1.4.1.1 Precipitation and Occult Precipitation**

As poikilohydric organisms, epiphytic lichens rely on moisture from precipitation, dew or mist. This is either intercepted by lichens directly, or reaches them *via* stemflow and throughfall. The relationship between amount of precipitation and epiphyte community structure is well documented, with some of the most striking examples being described from desert regions (Nash III & Moser, 1982).

Mean annual precipitation in the UK typically ranges from 400-3500 mm y<sup>-1</sup> (Met Office Data, 2009), with a gradient of increasing rainfall from east to west. This is due to the dual effects of a) an east-west gradient in oceanicity

and b) the predominance of higher ground in the west. An investigation by Wolseley *et al.* (2006<sup>a</sup>) identified rainfall as a key factor in lichen community composition in areas with a mean annual precipitation  $> 1500 \text{ mm y}^{-1}$ . The implication of this finding is that species identified as being acidophytic/nitrophobic in the wetter, western side of the UK might not be present in the drier east due to insufficient rainfall and not because of high  $[\text{NH}_3]$  values. Consequently, rainfall needs to be assessed as a potential confounding variable, and possibly some subjective decisions made regarding inclusion or exclusion of certain species in the bio-monitoring programme.

In addition to annual rainfall depth, precipitation chemistry can also directly affect lichen community composition. Although mean annual precipitation in Eastern England is lower than in Wales and Western Scotland, rainfall acidity is greater (NEGTAP, 2001). The lower pH of English rainfall means the  $[\text{NH}_3]: [\text{NH}_4^+]$  ratio is higher, based on shifts in the chemical equilibria. To further complicate this issue, the greater precipitation in the west means that N deposition is generally much higher than in the east. Other chemical components of rainfall might also be important in lichen community composition. One such element that has previously been identified as having a key role is S, and is discussed in more detail later (see Section 1.4.1.4).

In addition to annual rainfall, occult precipitation (i.e. wind driven fog and cloud) is also more frequent at high elevations, increasing water deposition rates. Because fog and cloud water droplets contain higher ionic concentrations

than rainfall in fog-free locations (Elias *et al.*, 1995; Fuhrer, 1986), occult precipitation enhances N and S deposition at high altitudes.

This effect is enhanced by the seeder-feeder mechanism. This works through the formation of orographic cloud or mist (by vapour condensation), which collects aerosols from lower down windward slopes of hills or mountains and acts as a “feeder” cloud. Any rain droplets falling from “seeder” clouds (located at higher altitudes), that pass through the feeder cloud, thus increase the concentrations of solutes deposited. The seeder-feeder mechanism typically occurs where the feeder clouds form, which is at altitudes between 300 and 1000 m in the UK (NEGTAP, 2001). Therefore, to avoid increased aerosol concentrations when undertaking the field study, selected sites should be at altitudes below this altitude.

#### **1.4.1.2 Altitude and Temperature**

Altitude also influences temperature in addition to precipitation and occult precipitation. In general, the regional temperature differences are probably too small to have a significant effect on epiphytic communities. A possible exception might be the differences between the Mediterranean elements along the south coast and lowland S.E. England and the areas of high altitude in N. Scotland. Given that sites will be surveyed at low altitude to avoid the influence of occult precipitation (see Section 1.4.1.1.), the effect of altitude-related temperature differences are likely to be mitigated and, therefore, temperature effects are not considered further in this project. However, the effects of temperature may need future evaluation in conjunction with



precipitation factors, given the potential impact of climate change and the possible shifts in community composition (Ellis *et al.*, 2007; van Herk *et al.*, 2002) that may arise in the future.

#### **1.4.1.3 Exposure to Sunlight (Irradiance and Aridity)**

Light is an influential factor in epiphyte ecology (James *et al.*, 1977), and it was considered by Wolseley *et al.* (2004) that shading could be a problem in the standardised use of epiphytes as bio-monitors. One source of shading variability is the use of different tree species during surveys, and support of this can be found in comparative studies by Loppi & Frati (2004) and Uliczka & Angelstam (1999). The canopies of different tree species cast shade to varying degrees of intensity, with differences between deciduous and evergreen species being particularly pronounced. Loppi & Frati (2004) studied the epiphytes of evergreen and deciduous trees in Central Italy and found that epiphyte diversity was 1.5 times greater on *Tilia platphyllos* than *Quercus ilex*. This finding is contrary to the commonly held belief that *Quercus* spp. typically have a greater SR. The reason for this was described as being the result of the evergreen *Q. ilex* having a more persistent shading effect. The possibility that variation among tree species in the form of shading affects epiphytic community structure clearly needs consideration.

#### **1.4.1.4 Sulphur Dioxide**

Although this study is focussing on relationships between lichens and N pollution (especially that of NH<sub>3</sub>), other pollutants have, and still do, influence epiphytic communities. These include heavy metals (Garty *et al.*, 1992) and

SO<sub>2</sub> (Gilbert, 1986; Gilbert, 1988; Hawksworth & Rose, 1970). Sulphur dioxide was previously a major atmospheric pollutant in the UK due to industrialisation, and the effect it had on epiphytic communities, both in its role as an acidifier of substrata and a toxin, is well documented (Hawksworth & Rose, 1970; Nimis *et al.*, 1990).

Sulphur dioxide was the dominant pollutant up to the 1980's. The dramatic UK-wide reductions in S that emissions that subsequently took place, e.g. from 3.26 Mt-S yr<sup>-1</sup> in 1970 to 0.60 Mt-S yr<sup>-1</sup> in 1999 (NEGTA, 2001), mean that this is no longer the case. But even if the direct influence of SO<sub>2</sub> on epiphytes is now a secondary concern compared to the impacts of N pollution, the effects of S pollution still requires consideration because of the long-term effects of SO<sub>2</sub> on trees over 40 years old and possible interactions with N pollution.

Ammonia accelerates the oxidation of SO<sub>2</sub> into SO<sub>4</sub><sup>2-</sup> in the aqueous phase (Junge & Ryan, 1958). Two important reactions can occur due to formation of SO<sub>4</sub><sup>2-</sup> in precipitation. The first is the well documented increased acidification of precipitation as H<sub>2</sub>SO<sub>4</sub> is formed (O'Neill, 1993). This notion is held out in the findings of the NEGTA Report (2001), where concentrations of NH<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and non-sea salt SO<sub>4</sub><sup>2-</sup> were all at their highest in the eastern part of Britain, which also had the most acidic precipitation. The second reaction involves the formation of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The formation of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> is a rapid process, and can form in the gaseous phase by the reaction between NH<sub>3</sub> and SO<sub>2</sub> on vegetation surfaces (van Breeman *et al.*, 1982) or in the aqueous phase in droplets (McKay, 1971; van Herk, 1999). Although [NH<sub>3</sub>], [NH<sub>4</sub><sup>+</sup>] and

$[\text{SO}_4^{2-}]_{\text{NSS}}$  are higher in the east of Britain, more  $\text{SO}_4^{2-}$  and  $\text{NH}_4^+$  is deposited in the west because of the higher mean annual precipitation. This effect is most prominent in upland western regions where high annual rainfall is complemented with occult precipitation (NEG-TAP, 2001).

The greater deposition of  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  in upland western regions can result in increased acidity of soils and bark surfaces. The effect of  $\text{SO}_4^{2-}$  in this can be direct when it is deposited as  $\text{H}_2\text{SO}_4$  due to proton donation; and acidification by  $\text{NH}_4^+$  deposition can occur through the nitrification of the compound (Killham, 1994; van Breeman *et al.*, 1982). Consequently, if the bark pH or that of the epiphyte is a critical factor in epiphyte community composition, then the high deposition of  $(\text{NH}_4)_2\text{SO}_4$  in some parts of western Britain where  $[\text{NH}_3]$  values are close to those in parts of the east, might help buffer the negative impacts of  $[\text{NH}_3]$  on the acidophytic established there.

#### **1.4.1.5 Geology, Pedology and Dust**

The effect of geology and soils on corticolous species is less direct than for saxicolous species. It typically occurs *via* deposition of windblown dust originating either directly from surrounding rock or indirectly from the soil. The exposure of corticolous communities to calcareous dusts has been identified as being important in the development of nitrophytic communities. For example, Benfield (1994) observed that *Quercus* trees exposed to dust support similar lichen communities as those exposed to fertilisers and other farming activities. Deposition of dust from limestone quarries increases bark pH (Loppi *et al.*, 1997; Gilbert, 1976). However, in an assessment of quarry dust effects at a Mediterranean location, Loppi & Pirintsos (2000) found that

both acidic and basic dust had similar effects on lichen communities, and that distance from the quarry had the greatest influence on community composition. This work did not measure the effect of different dust types on bark pH, so it is not possible to compare the effect of dust with that of  $\text{NH}_3$  as a pH modifier, despite apparent effects on epiphytic communities being similar. But an assumption that acidic and basic dust would affect bark pH differently would imply that this is the case. Nonetheless, it seems clear that dust is a confounding variable when trying to assess a bio-monitoring scheme for  $\text{NH}_3$ .

A second possible vector of ion transport from soils to bark surface is *via* root uptake and transport through the trees vascular system. The influence of soil type on epiphytic communities has been largely overlooked in the literature. This might be because much of the previous work focussed on epiphytes on tree boles rather than in the canopy, and it is the latter that have been recently shown to have strong relationships with the soil (Benner *et al.*, 2007; Kermit & Gauslaa, 2001). Nonetheless, a study undertaken by Gustafsson & Eriksson (1995) found that positive correlations existed between soil exchangeable  $\text{Ca}^{2+}$ , total bark  $\text{Ca}^{2+}$  and bark exchangeable  $\text{Ca}^{2+}$ .

Urbanisation generates dust from roads, buildings and building materials (Fuller & Green, 2004; Gilbert, 1976). A cumulative effect of road dust has been observed both on terrestrial (Myers-Smith *et al.*, 2006) and epiphytic (Marmor & Randlane, 2007) vegetation. In both of these instances an increase in bark pH occurred where it was initially acidic in nature. This effect in urban environments is not likely to be a significant factor in the present study as

priority was given to rural survey sites. However, to help offset complications arising from the other factors mentioned, sites located near quarries or on calcareous soils were excluded in the study.

#### **1.4.1.6 Site History**

Past habitat structure and land management can have a great influence on current plant communities, including lichens (Aude & Poulsen, 2000; Gu *et al.*, 2001; Josefsson *et al.*, 2005; Wolseley *et al.*, 2006<sup>b</sup>). Woodland plantations are a common feature in the UK landscape, and many in the Pennines, Scotland and Wales were established in what was previously open moorland. This dramatic change in habitat means that many of the species expected to be present in a forested area may not be found due to a history of limited propagule availability. The often depauperate species pool of epiphytes in isolated plantations might not provide a reliable indication of air quality. Care should therefore be taken during site selection to ensure that historically unsuitable locations are avoided. This problem can be alleviated by selecting designated sites or semi-natural habitats (e.g. Sites of Special Scientific Interest) as these are likely to possess species that are more representative of the natural habitat species pool (Beleya & Lancaster, 1999; Keddy, 1992; Kelt *et al.* 1995). However, it is also worth noting that where habitat connectivity is disrupted, epiphyte dispersal could also be affected much like other organisms (Virtanen & Oksanen, 2007; Berglund & Jonsson, 2005).

#### **1.4.1.7 Aspect and Inclination**

Aspect and inclination of tree boles affects exposure to light, and can also influence other factors important to epiphyte ecology. The more exposed

aspects will experience higher temperatures and greater aridity than the more sheltered aspects. The prevailing UK weather fronts typically originate from the southwest, and these aspects of tree boles are also more exposed to the associated conditions than the northern and eastern faces. This further compounds microclimatic variation, which can influence species composition around the bole. Similarly, the degree of trunk inclination can modify the distribution of stemflow.

Examples of the way in which epiphytic species are affected include the preponderance for many bryophytes to colonise the sheltered north-facing aspect of tree trunks (which are typically more humid and shadier) while the lichen *Cliostomum griffithii* displays a preference for drier, more exposed surfaces. During survey work it will be possible to account for localised aspect and inclination effects by standardising trees selection to those with relatively straight boles and by surveying all four trunk aspects as described by [Asta \*et al.\* \(2002\)](#).

#### **1.4.1.8 Bark Topography**

Differences between tree species in bark topography can cause commensurate epiphytic variation in community composition. This can be seen, for example, when comparing *Acer pseudoplatanus* and *Fagus sylvatica* against phorophytes such as *Populus tremula* and *Quercus robur*, both of which have considerably more rugose bark. The microclimate formed in recesses between the ridges of rough barked trees provides a habitat for lichen species such as *Calicium viride*. Rough bark also provides a greater opportunity for thallus fragments of

foliose and fruticose lichens (e.g. *Ramalina farinacea* and *Usnea subfloridana*) to become lodged in its surface.

In contrast, some smooth-bark (e.g. *Fagus sylvatica* and *Tilia x cordata*) can be thin, making the bark temperature sensitive and prone to the stress of dessication (Jönsson, 1998). The effect of these factors means that some smooth-barked trees typically do not support diverse or rich epiphytic assemblages. As the epiphytic diversity on the trunks of smooth-barked trees can be considerably lower in comparison to rough-barked trees, they might not provide as good a representation of the species pool in a given region compared to those on rough-barked trees.

#### **1.4.1.9 Bark Sloughing**

Studies of epiphytic community dynamics on trees (Ellis & Coppins, 2007; James *et al.*, 1977) highlight the need to consider bark exfoliation as a determining factor. In order for a lichen to maintain a population on a tree with exfoliating bark, its growth rate would need to be at least on par with the rate of bark exfoliation. This exfoliation can range from the peeling of the thin, papery bark of *Betula* spp. to the continuous flaking of bark observed in *Larix* and *Pinus* spp.

The rate of bark sloughing can also vary depending on the health of a tree. For example, canker caused by the fungus *Hypoxylon atropunctatum* attacks the vascular system and kills the cambium, causing bark sloughing and tree death. The result of this attack is the permanent loss of bark and the associated

corticolous epiphytes, creating a habitat for lignicolous species. To compensate for this, only healthy-looking trees should be surveyed.

#### **1.4.2 Biotic Factors**

##### **1.4.2.1 Bark Age**

Differences in substratum age, and hence the time it has been available for colonisation, can be reflected in the successional patterns of epiphytes (Ruchty *et al.*, 2001; Section 1.4.2.5). Age-related physicochemical variation (e.g. shading, bark topography and pH), can also occur in a single species and influence lichen colonisation (Lewis, 2007; Wolseley & James, 2000). Legrand *et al.* (1996) identified changes in bark pH along the trunks of *Abies pectinata* and *Picea abies*, and suggest that it might be a result of variation in exposure time to leaching and stemflow, and also linked to changes in bark thickness along. Note that stemflow effects are likely to be more noticeable on trunks. Support for this comes from Kermit & Gauslaa (2001) who reported that acid rain had a negligible on the pH of twigs, and that intra-species differences in twig pH were likely due to small-scale variations in soil chemistry.

##### **1.4.2.2 Bark pH**

Bark pH is an important ecological determinant of epiphytic lichen community structure and one that is determined by many of the previously discussed factors, including NH<sub>3</sub> and SO<sub>2</sub> pollution, tree species, dust impregnation and substratum age (Barkman, 1958; Gilbert, 1976; Jüriando *et al.*, 2009; Legrand *et al.*, 1996; van Herk, 2001; Wolseley *et al.*, 2006<sup>a</sup>). Henriksson (1958) showed that the optimum pH for mycobiont spore development in *Collema*



*tenax* was 7.5, and growth of the species was best between pH 5.6-7.4 (Henriksson, 1964). One possible reason for a disparity in pH preferences between establishment and growth of lichens is the specific preferences of the different bionts comprising the thallus. Yoshimura *et al.* (1987) studied the growth rates of the symbionts of *Cladonia vulcanii* in culture and found that the photobiont had a higher optimum pH than the mycobiont. However, both bionts had the same growth rate at pH 5.5, which was the typical soil pH where the species was found. The authors concluded from this that the successful formation of a lichen thallus requires an equal growth ratio for both bionts, and that this is determined by pH. This explanation is consistent with lichen colonisation failure being due to changes in substratum pH.

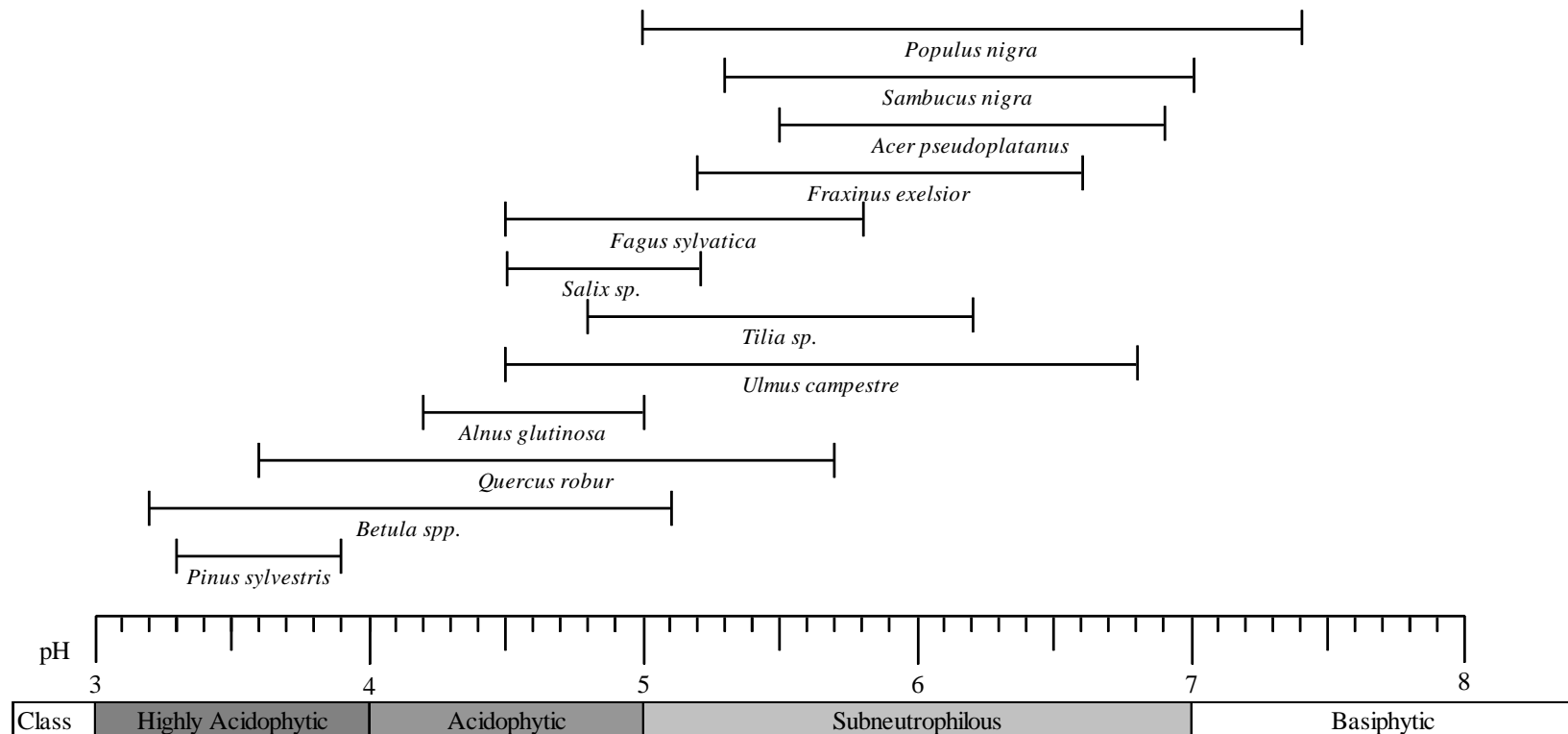
Field observations also pointed to the importance of substratum pH on lichens, such as the positive association between high bark pH and cation levels and the occurrence of *Lobaria* spp. (Wolseley & James, 2000; Bates, 1992; Farmer *et al.*, 1991). Wolseley & James (2000) concluded that small decreases in bark pH due to previous SO<sub>2</sub> pollution, was a likely reason for the failure of new *L. pulmonaria* thalli to successfully colonise and develop.

Bark pH of phorophytes is a key factor to consider when selecting trees to survey for bio-monitoring purposes. There is natural inter-specific variation in tree bark pH so that tree species can be ranked or grouped into different pH classes (Figure 1.1). The bark surface pH of a given tree species can vary for many reasons, which have been discussed previously. Atmospheric N (Section 1.2.3) and S (Section 1.4.1.4) pollution play a role in perturbing bark pH. The

effect of pollutants on bark pH can vary between tree species. For example, the acid-barked species *Pinus sylvestris* and *Quercus robur* are noticeably affected by pH-raising pollutants, whereas the sub-neutral *Tilia cordata* is not (Marmor & Randlane, 2007; Wolseley *et al.*, 2006<sup>a</sup>). Despite this, acid barked phorophytes are more appropriate for bio-monitoring, as those possessing sub-neutral bark naturally favour nitrophytic epiphytes on their trunks and rarely possess acidophytes in dry climatic conditions (Wolseley *et al.*, 2006<sup>a</sup>). The ideal approach for establishing a bio-monitoring scheme for NH<sub>3</sub> using epiphytic lichens would be to survey lichens on one tree species, but this is probably not practical on a UK-wide scale.

#### **1.4.2.3 Grazing**

The chance of localised or regional epiphytic extinction purely through to extensive grazing is very low, and lichens have been shown to possess defensive mechanisms to counter the effects. For example, restrictions to lichen grazing by the molluscs *Cochlodina laminata* and *Pallifera varia* on *Lobaria pulmonaria* and *L. scrobiculata* have been attributed to carbon-based secondary compounds (Lawrey, 1983; Vatne *et al.*, 2011). Experimental work by Asplund (2011) supports this suggestion, as thalli with their secondary compounds removed were shown to be intensively grazed compared to untreated thalli. Consequently, grazing effects were not factored into this study as it was deemed impractical given the current scope of the project. However, its effect as a cumulative ecological pressure should be acknowledged.



**Figure 1.1** Classification of trees based upon bark pH. Tree bark pH varies naturally according to species. From this observation different tree species can be grouped together into four classes (highly acidic, acidic, subneutral and basiphytic) based on the typical range of their pH values. Source: Barkman (1958).

#### 1.4.2.4 Competition

Lichens are considered to have a low competitive ability and are subsequently classified as being pioneer species (Grime 1977; Topham, 1977). Despite this, the issue of competition between epiphytes should be given consideration as epiphytic lichens show non-random associations. The associations observed between epiphytic cryptogams are potentially a consequence of mutualistic or competitive effects, and where competition is possibly occurring, an inevitable hierarchy of competitive dominance exists between species (John & Dale, 1995; Pentecost, 1980). This was observed by John (1992) in the inter-thalline interactions between the heavier *Flavoparmelia caperata* and the lighter, more delicate *Melanelixia subaurifera* where the thalli of *F. caperata* displayed a preponderance to overlap those of *M. subaurifera*. Thus, competition can play an influential role in determining species composition between epiphytic species.

Competitive interactions between lichens and bryophytes are even less clear. Some bryophytes (e.g. *Frullania dilatata*, *Isothecium myosuroides* and *Eurynchium praelongum*) have a preponderance to out-compete lichens, particularly foliose and sexually reproducing crustose lichen species, in moist, shaded conditions due to their typically faster growth rate and capacity to grow over lichens. However, some crustose (e.g. *Haematomma* spp. *Ochrolechia* spp. and *Pertusaria albescens* var. *corallina*) and leprose lichens (e.g. *Lepraria* spp.) grow over bryophytes (Personal observations; Topham, 1977).

Although patterns of competition have been reported, such as the previously described observations of [John & Dale \(1992\)](#), care should be taken when trying to interpret these findings as competition and facilitation are two different processes that are closely linked. For example, the positive associations between bryophytes and the lichens *Cladonia* spp., *Normandina pulchella* and *Bilimbia sabuletorum* ([Jüriando et al., 2009](#); [John & Dale, 1992](#)) suggest that species associations are also likely to be due to facilitation (see Section 1.4.2.5).

Competitive exclusion should not be ignored as a potential influence, especially in areas where past perturbation through [SO<sub>2</sub>] pollution has not occurred. But the absence of species alone cannot be accepted as proof of competitive exclusion, as it may merely be the result of unsuitable microclimate. Recent studies assessing the effect of NH<sub>3</sub> pollution on epiphytes indicate that interspecific competition between lichens is not a significant factor in relation to bio-monitoring for NH<sub>3</sub>. This is because acidophyte loss was observed at lower NH<sub>3</sub> values than nitrophytic colonisation ([Wolseley et al., 2005<sup>a</sup>](#); [Wolseley et al., 2006<sup>a</sup>](#)). The suggestion that competition is not an influential factor is supported by other studies on macrolichens in relation to phorophyte species and age ([Uliczka & Angelstam, 1999](#)). Consequently, competitive interactions are not factored into the analysis for the bio-monitoring scheme.

#### 1.4.2.5 Succession

Succession is a temporally driven factor and positive relationships have been found between tree age and epiphyte abundance ([Uliczka & Angelstam, 1999](#)), with evidence of successional trends in epiphyte colonisation identified on several tree species ([Hilmo \*et al.\*, 2009](#); [Lewis & Ellis, 2010](#)). It is therefore evident that older bark offers a geographical consistency enabling greater completion of processes linked to succession, such as facilitation. Although the trunks of older trees (theoretically near the latter end of successional development) might support greater numbers of epiphytic species, the transient nature of species colonisation and persistence means that early colonising species, such as some sexually reproducing crustose lichens ([Ellis & Coppins, 2007](#)), might not be present at a later point in time.

Another issue arising from surveying tree boles is that the presence of some species on trunks might partly reflect earlier atmospheric conditions, and consequently bias the bio-monitoring scheme. This is due to natural time lags between perturbation and response time of a given species ([Metzger \*et al.\*, 2009](#)). An example of this can be seen where an influential factor (e.g. bark pH) affects the colonisation and development of lichen thalli (see Section 1.4.2.2). In this instance the current species population may not be adversely affected in terms of its presence, even though colonisation and development of future generations is prevented. As a result, an extinction debt occurs whereby persistence of older thalli may complicate any attempt to evaluate current conditions.

In an attempt to resolve problems relating to the extinction debt, species lost due to the natural dynamics of community composition (facilitation and competition) and the influence of past site perturbation, branch surveys were also undertaken. Surveying branches has previously proved to be a reliable means of evaluating current conditions and recent changes to a site (Gadsdon *et al.*, 2010; Larsen Vilsholm *et al.*, 2009; Wolsleley *et al.*, 2006<sup>a</sup>; Wolseley & Pryor, 1999). However, branches can possess different lichen assemblages to trunks, particularly with respect to the microlichen species. This further emphasises the need to identify a list of indicator species for the UK, and if possible, to formulate a list that can be applied to both trunk and branch survey techniques.

## **1.5 Aims and Objectives of the Present Work**

The aim of the present project was to identify a suite of key indicator species for NH<sub>3</sub> pollution among epiphytic lichens and bryophytes, and to use these to develop a quantitative bio-monitoring scheme that can be used to indicate NH<sub>3</sub> pollution on a local scale within the British Isles. This involved achieving the following specific objectives.

1. To select field sites throughout the UK, which experience different background levels of NH<sub>3</sub>.
2. To quantify the abundance of epiphytic lichens and bryophytes at selected field sites.
3. To use quantitative methods to test for appropriate nitrophilic and nitrophobic indicator species with respect to NH<sub>3</sub> concentrations.

4. To develop an optimum epiphyte scoring system to indicate  $\text{NH}_3$  concentrations.
5. To summarise and evaluate climatic and environmental chemical data (both modelled and measured) for all sites in order to identify factors that co-vary.
6. To examine relationships between the non- $\text{NH}_3$  based variables, both climatic and environmental chemistry, and the epiphytic scoring system to identify confounding variables.
7. To develop a regression model in which epiphyte scores are related to  $\text{NH}_3$  and other key determinants (confounding variables).
8. To identify the effects of individual co-varying environmental factors on epiphytes by analysing data from a controlled field N-enrichment experiment.
9. To seek evidence of physiological differences between nitrophobes and nitrophiles under laboratory conditions.

This work tests the following hypotheses.

1. Epiphytic lichen and bryophyte communities are quantitatively related to  $\text{NH}_3$  concentrations in the local area.
2. Different component N forms in deposition have contrasting effects on substratum pH.
3. Nitrophobes and nitrophiles have contrasting physiologies, with nitrophobes having a lower pH optima for, and higher rates of, phosphomonoesterase activity compared to nitrophiles.



## **Chapter 2. Regional-Scale Survey Methods**

### **2.1 Introduction**

This chapter explains the selection process for sampling sites for the regional-scale survey, together with the biological and chemical methods applied. The approach of the survey is based upon the methods previously used in an extensive UK study into lichen diversity relationships with  $\text{NH}_3$  concentrations, and a comparative assessment of the methodology as used by experts and non-experts (Wolseley *et al.*, 2005<sup>b,c</sup>). A refinement of these methods was undertaken to evaluate the influence of potentially confounding factors.

In addition, an attempt was made to validate use of the Lichen Acidophyte Nitrophyte ( $L_{AN}$ ) scoring scheme by testing it against the currently established indicator species lists (Wolseley, 2005; Wolseley *et al.*, 2009; OPAL, 2011) and a revised list generated by this study that, if relevant, would include bryophytes. By collating a wide range of data on community composition, atmospheric pollutants, substratum pH values and meteorological variables regionally across the UK, this project aimed to formulate UK-specific lists of nitrophobic and nitrophilic indicator species. These would then be used to establish a nationwide bio-monitoring programme for atmospheric  $\text{NH}_3$  pollution for use by environmental and conservation organisations.

## **2.2 Criteria for Site Selection**

A total of 28 sites were selected across the UK for the survey from an original shortlist of 150 potential sites (Appendix A). Site selection was established on a suitability criteria based on climatic and air chemistry variables, tree suitability, geology, accessibility and the possibility to undertake regular monthly monitoring of [NH<sub>3</sub>] at the site where required (Section 2.5.1-2.5.4).

### **2.2.1 Climatic and Air Chemistry Variables**

The survey sites were spatially distributed across as wide an area of the UK as possible in terms of both longitude and latitude. The goal of this approach was to account for the climatic and environmental variability found across the country. A brief review of UK climate and pollutant variables was initially undertaken to provide an insight into their spatial distribution across the UK, with the aim of identifying key regions to target for surveying. This was done by assessing Met Office climate maps (Met Office, 2008) for climate and from literature and web-based databases for air chemistry concentrations and deposition values (NEGTA<sup>P</sup>, 2001; Sutton *et al.*, 2004; Leith *et al.*, 2005<sup>b</sup>; CEH, 2008).

Where possible, survey sites were located within 1 km of National Ammonia Monitoring Network (NAMN) and Acid Gas and Aerosol Network (AGANet) monitoring stations. These provided measured data for nitrogen and sulphur compounds and base cations in air and precipitation relevant to the sites. This approach did not prove possible for all the survey locations for a number of reasons (Appendix A), most notably:

- a) inability to obtain permissions and access rights to many of the sites
- b) lack of suitable tree species in proximity to the monitoring stations
- c) insufficient environmental variables measured at certain sites.

To offset this problem, NH<sub>3</sub> monitoring stations were established at those sites where no NAMN monitoring was being undertaken to supplement the measured data obtained from sites that were part of the NAMN (Section 2.5). At these sites, the availability of a volunteer willing and able to act as a site operator for the monthly maintenance of the station was required as an additional criterion to site selection. Data for the remaining environmental variables were acquired from modelled sources (Section 2.6).

### **2.2.2 Landscape Factors**

Sites with a history of continuity, such as designated sites with relevant conservation interest, were included in the survey where possible. However, this was not always achievable due to other site selection criteria not being met, limited feedback from many site operators and difficulties in obtaining permissions for sites.

Further limitations to site selection were imposed based upon geology and location. Calcareous woodlands were omitted from the survey to prevent the complications that deposition of base-rich soil dust upon the tree trunks would have upon substratum pH, and subsequently epiphytic species composition. Coastal woodlands and sites situated at altitudes exceeding 500 m were also

excluded. This was due to the influence of occult precipitation and oceanic  $\text{SO}_4^{2-}$  deposition, which, being greater at these locations (Choularton *et al.*, 1988; Fowler *et al.*, 1988; Inglis *et al.*, 1995), would compromise  $\text{SO}_4^{2-}$  data acquired. Locations within 50 m of a roadside or track known to carry vehicles were also omitted to minimise the influence of vehicular-based point source emissions of  $\text{NH}_3$  and  $\text{NO}_x$  on the community composition of epiphytes (Cape *et al.*, 2004).

### **2.2.3 Tree Availability**

Suitable survey sites were required to include acid-barked trees (Section 2.8) in close proximity ( $\bullet$  1 km) to each  $[\text{NH}_3]$  monitoring station. This criteria was established to account for the rapid dispersal of atmospheric  $\text{NH}_3$  (Fowler *et al.*, 1998; Sutton *et al.*, 1998), and evaluated through measurements of tree distances from monitoring stations at each site (Section 2.8.1).

## **2.3 Climate Data**

A comprehensive Met Office climate dataset for the 30-year period covering 1977-2006 inclusive was obtained at the 5 km resolution courtesy of Dan Chapman (CEH, Edinburgh). The mean values of 10 climatic variables were derived from these datasets: mean annual precipitation ( $\text{mm yr}^{-1}$ ) (PPN), mean annual rain days  $> 1$  mm ( $\text{RD} > 1$  mm), mean annual rain days  $> 10$  mm ( $\text{RD} > 10$  mm), mean annual temperature (T), mean annual minimum temperature ( $T_{\min}$ ), mean annual maximum temperature ( $T_{\max}$ ), mean monthly minimum temperature ( $T_{\min}$ ), mean monthly maximum temperature ( $T_{\max}$ ),

mean annual growing degree days > 5 °C (GDD) and mean monthly growing degree days > 5 °C (GDD).

### **2.3.1 Oceanicity**

An oceanicity index (OI) was calculated for each survey site based upon the method described by Averis *et al.* (2004). The primary purpose was to try and identify any influence that levels of oceanicity had upon epiphytic species composition (Wolseley *et al.*, 2006<sup>a</sup>). The secondary purpose of the OI was to reduce the number of climatic effects used in future analyses by integrating  $T_{\min}$ ,  $T_{\max}$  and RD into the single value of the OI (Eq. 2.1). Given the naturally occurring climatic variability, the OI was calculated based upon the 30-year means of rain days and temperature data from 1977-2006 inclusive.

$$OI = RD > 1 \text{ mm} / (T_{\max} - T_{\min}) \quad [\text{Eq. 2.1}]$$

### **2.3.2 Preliminary Analysis of Climatic Variables**

Given the large number of variables held relative to the number of sites surveyed, a means of consolidating the climatic variables was sought. Based on previous work identifying the influence of oceanicity on lichen species distributions across the UK (Wolseley *et al.*, 2006<sup>a</sup>), the OI was evaluated against the other climatic variables at each site. Spearman's Rank Correlations (SRC) were undertaken to validate the exclusion of variables closely correlated to the OI from the subsequent ordination analysis (Section 3.2.1). This non-parametric approach was used as data were non-normally distributed and attempts at transformations were not successful. Climatic variables with good

correlations to the OI (subjectively set at  $r_s \bullet 0.70$ ) were duly removed from future analysis, and the OI used as a representative value for the excluded variables. Climatic variables that displayed a lower ( $r_s < 0.70$ ) correlation were included in analyses independently from the OI.

## **2.4 Measured Environmental Variables**

Measured concentration data for environmental variables at survey sites that were derived from the NAMN and/or AGANet monitoring networks were acquired from the UK Pollutant website (DEFRA, 2010). Only a subset of the survey sites selected for this study were part of the NAMN and/or AGANet (Table 2.1) because many of the sites were unsuitable for epiphyte surveying (Appendix A).

It was not practically feasible to establish monitoring stations for all the assessed environmental variables at those sites which were not part of either the NAMN or AGANet networks. Therefore a decision was made to prioritise the monitoring of  $\text{NH}_3$  at such sites to enable a more effective calibration of  $\text{NH}_3$  concentrations with the bio-monitoring scheme scores (Chapter 5). Ammonia was also prioritised due to its greater spatial variability compared to other pollutants (Hallsworth *et al*, 2010; van Pul *et al.*, 2009), which would result in modelled estimates of the gas being less robust than those of other pollutants, such as aerosols and  $\text{SO}_2$ .

## 2.5 **NH<sub>3</sub> Monitoring**

The NAMN utilises two systems for atmospheric NH<sub>3</sub> capture across the UK: Adapted Low-cost Passive High-Absorption (ALPHA) samplers (Tang *et al.*, 2001) and a DEnuder for Long-Term Atmospheric sampling (DELTA) diffusion denuder system (Sutton *et al.*, 2001). Both of these methods were operational at some of the survey sites.

Monthly mean [NH<sub>3</sub>] values were measured at all of the 28 survey sites. [NH<sub>3</sub>] data were obtained by a combination of ALPHA samplers and DELTA denuders from the NAMN for 18 of the survey sites (DEFRA, 2011; DEFRA, 2010), and by Gradko diffusion tubes from the Environmental Change Biodiversity Network (ECBN) at one site courtesy of Maggie Hatton-Ellis from the Countryside Council for Wales. At eight of the remaining survey sites that were not part of an established monitoring network, monitoring stations were installed from August 2009. ALPHA samplers were used for this purpose rather than DELTA denuders as they did not require a power source to operate them, and were easier to maintain. The remaining site (Hinderclay Fen) was originally part of the NAMN, but was one of numerous sites deleted from the network in 2010 due to financial cutbacks. To compensate for this, an ALPHA sampler monitoring station was also established at this site for continuity, with measurement commencing from January 2010 (Table 2.1).

**Table 2.1** Details of concentration data for environmental variables at the 28 UK-wide survey sites. The terms [N] and [S] indicate that a comprehensive set of nitrogen and sulphur compounds (comprising  $[\text{NH}_3]$ ,  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$ ,  $[\text{HNO}_3]$ ,  $[\text{SO}_2]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ) were monitored at that given site.

Site Name	Site Code	Region	OS Grid Ref	Pollutant Network	Pollutants Measured	NH <sub>3</sub> Monitoring Method	NH <sub>3</sub> Monitoring Period
Alice Holt	AH	Hampshire	SU 805 427	NAMN	$[\text{NH}_3]$	ALPHA sampler	Sep. 1996 - Feb. 2009
Banchory	Ba	Aberdeenshire	NO 676 985	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Bretton Lakes LNR	BL	West Yorkshire	SE 282 123	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Brown Moss SAC	BM	Shropshire	SI 563 390	NAMN	$[\text{NH}_3]$	Denuder	May. 1998 - Dec. 2009
Bush Estate	BE	Midlothian	NT 245 635	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Oct. 1996 - Mar. 2010
Correl Glen NNR	CG	Co. Fermanagh	IH 075 544	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Oct. 1996 - Mar. 2010
Cymystwyth	Cw	Ceredigionshire	SN 771 742	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Feb. 1997 - Mar. 2010
Dunalastair Estate	DE	Perthshire	NN 748 600	NAMN	$[\text{NH}_3]$	ALPHA sampler	Aug. 2000 - Mar. 2010
Glen Nant NNR	GN	Argyll & Bute	NN 013 280	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Glenmore Wood ASSI	GM	Co. Tyrone	IH 651 609	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Grizedale Forest	GF	Cumbria	SD 337 941	NAMN	$[\text{NH}_3]$	ALPHA sampler	Aug. 2000 - Mar. 2010
Hinderclay Fen	HF	Suffolk	TM 025 787	None*	$[\text{NH}_3]$	ALPHA sampler	Jul. 2006 - Jul. 2010
Mere Sands Wood LNR	MS	Cheshire	SD 447 157	NAMN	$[\text{NH}_3]$ & $[\text{NH}_4^+]$	Denuder	Sep. 1996 - Dec. 2009
Moninea Bog ASSI	MB	Co. Fermanagh	IH 301 217	None*	$[\text{NH}_3]$	ALPHA sampler	Sep. 2009 - Jul. 2010
North Wyke	NW	Devon	SX 659 983	NAMN	$[\text{NH}_3]$ & $[\text{NH}_4^+]$	ALPHA sampler	Sep. 1996 - Mar. 2010
Plas y Brenin	PB	Gwynedd	SH 716 578	NAMN & AGANET	[N], [S] & [base cations]	Denuder	May. 2006 - Dec. 2009
Rannoch Forest	RF	Perthshire	NN 603 533	NAMN	$[\text{NH}_3]$	ALPHA sampler	Aug. 2000 - Dec. 2009
Redgrave & Lopham Fen NNR	RL	Suffolk	TM 053 802	NAMN	$[\text{NH}_3]$	ALPHA sampler	Dec. 1996 - Mar. 2010
Rothamsted	Ro	Hertfordshire	TL 123 129	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Sep. 1996 - Mar. 2010
Ruabon NNR	Ru	Wrexham	SI 225 489	NAMN	$[\text{NH}_3]$	ALPHA sampler	Nov. 1996 - Mar. 2010
Strath Vaich Dam	SV	Highlands	NH 348 750	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Sep. 1996 - Mar. 2010
Sutton Bonnington	SB	Nottinghamshire	SK 505 268	NAMN & AGANET	[N], [S] & [base cations]	ALPHA sampler	Dec. 1996 - Mar. 2010
Twentywellsick Wood LNR	Tw	South Yorkshire	SK 326 808	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Tycanol Wood NNR	Ty	Cardiganshire	SN 090 366	ECBN	$[\text{NH}_3]$	Diffusion tubes	Mar. 2009 - Mar. 2010
Upper Ballinderry River ASSI	UB	Co. Tyrone	IH 765 787	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Wood of Cree SSSI	WC	Dumfries & Galloway	NX 386 716	None*	$[\text{NH}_3]$	ALPHA sampler	Aug. 2009 - Jul. 2010
Wytham Wood SSSI	WW	Oxfordshire	SP 462 082	NAMN	$[\text{NH}_3]$	Denuder	Oct. 1996 - Dec. 2009
Yarner Wood NNR	YW	Devon	SX 786 789	NAMN & AGANET	[N], [S] & [base cations]	Denuder	Sep. 1996 - Mar. 2010

ASSI = Areas of Special Scientific Interest; LNR = Local Nature Reserve; NNR = National Nature Reserve; SAC = Special Area of Conservation; SSSI = Site of Special Scientific Interest

\*  $\text{NH}_3$  concentration in air was measured at these sites as part of this thesis.



The correlation between  $[\text{NH}_3]$  derived from ALPHA sampler and DELTA denuders has been compared using data from NAMN inter-comparison sites where both methods were operated simultaneously (Tang *et al.*, 2007). A good relationship was shown to exist between the two methods, indicating that an assumption can be made that  $\text{NH}_3$  concentration data obtained from the NAMN would be relatively consistent, despite the two different monitoring systems.

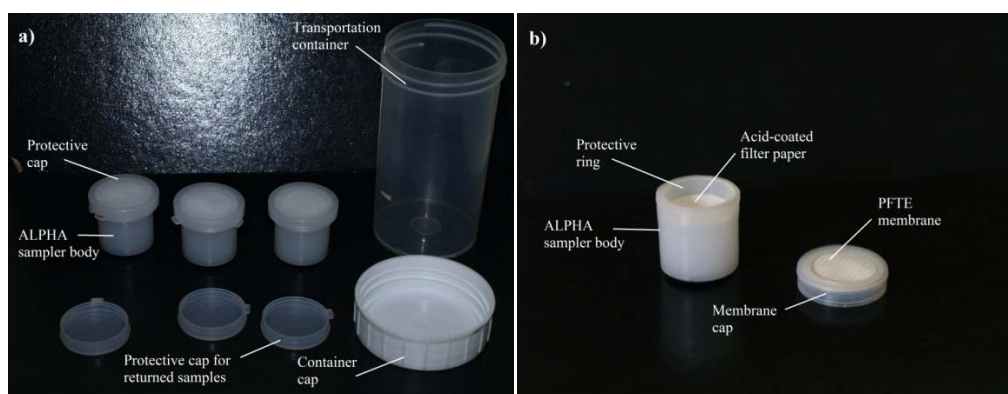
### **2.5.1 ALPHA Sampler Preparation**

Nitrile gloves were worn during every phase of ALPHA sampler preparation and analysis to prevent contamination. Seven to eight Swiftlab<sup>®</sup> grade 604 24 mm diameter cellulose filter papers were evenly placed into sterile Petri dishes and 55  $\mu\text{l}$  aliquots of 12% solution of citric acid in methanol were added quickly to the centre of each filter paper. Once the solution had soaked through to the edges of each filter paper, the acid-coated filter papers were transferred to a dessicator attached to a KNF Lab Laboport<sup>®</sup> vacuum pump and dried for 3 minutes.

Once prepared, the acid-coated filter papers were placed inside the body of an ALPHA sampler and secured with a 1.5 mm thick plastic ring (Figure 2.1). Each ALPHA sampler body was then covered with a membrane cap incorporating a 0.6 mm PTFE membrane. This construction was then sealed with a protective cap to protect the membrane during transport of the ALPHA samplers to each monitoring site. The protective cap was installed to prevent both damage and accidental  $\text{NH}_3$  contamination of the acid-coated filter paper

during transportation. The ALPHA samplers were sealed around the lids with parafilm, sealed in a transportation container and kept in dark cold storage at 4°C until deployment in the field and, on return until analysis of the complete batch of ALPHA samplers for each given month. A control set of acid-coated filter papers were produced for each monthly batch of ALPHA samplers issued to the relevant sites. The production of the controls followed that described above. The [NH<sub>3</sub>] recorded for the control samples were used to correct the final atmospheric [NH<sub>3</sub>] obtained from the exposed filter papers established at each monitoring site (Section 2.5.4).

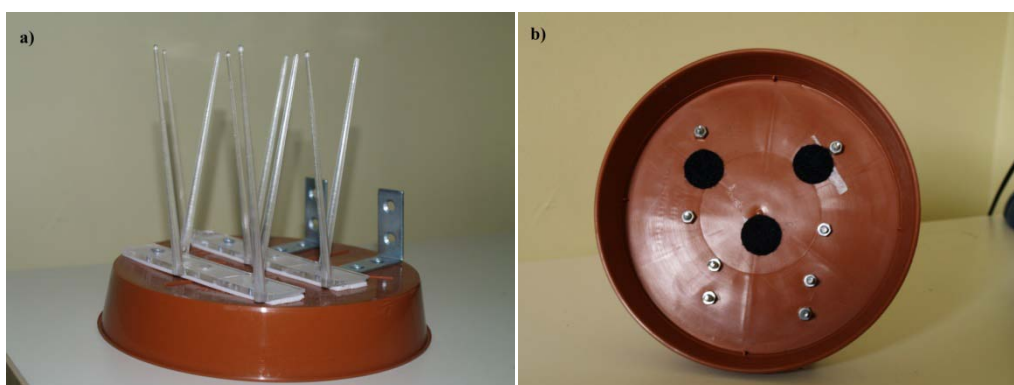
All unused acid-coated filter papers made during the production process were kept as spares as insurance against non-receipt of ALPHA samplers sent to monitoring site operators by post. Spare acid-coated filters were stacked inside a small sterile petri dish, sealed with parafilm, placed inside an airtight plastic container that was lined with acid-treated blotting paper, and stored in a refrigerator at 4°C. All spare filter papers were discarded after two months.



**Figure 2.1** ALPHA sampler construction for the monitoring of [NH<sub>3</sub>] in air. Three replicate ALPHA samplers (a) were issued to nine of the survey sites each month with an acid-coated filter paper (b) contained within each sampler.

### 2.5.2 ALPHA Sampler Installation

Three replicate ALPHA samplers were installed at each of the nine sites. These were attached to the underside of a 15 cm diameter plastic plant saucer using velcro tabs. The saucers were positioned upside down at 1.5 m above ground level using a metal pole and rail bracket to prevent contamination by  $\text{NH}_3$  in deposition. As an additional precaution, bird deterrents were added to the saucers to prevent birds from using them as perches, and thus, causing unnaturally high  $[\text{NH}_3]$  around the monitoring post (Figure 2.2).



**Figure 2.2** Hood of an ALPHA sampler monitoring station with attached bird deterrents (a) and velcro tabs on the underside (b) for the attachment of the ALPHA sampler badges.

The ALPHA samplers were changed on a monthly basis from 01/08/2009 to 01/07/2010 inclusive. The time and date that ALPHA samplers were changed each month at each site were recorded on a sheet by site operators (Appendix B), and used to calculate atmospheric  $[\text{NH}_3]$  (Section 2.3.4). All ALPHA samplers returned by site operators were stored at  $4^\circ\text{C}$  prior to analysis.

### **2.5.3 ALPHA Sampler Analysis**

Each ALPHA sampler was carefully dismantled under laboratory conditions, the exposed filter papers placed into 10 ml capacity vials, and 3 ml of deionised water added to each pot before being sealed with lids. The exposed filter papers were then left to soak for a minimum of 1 hour in the deionised water, and the pots shaken intermittently, to extract the chemical species contained within them.

Once the soaking period had elapsed, each filter paper was agitated in the extractant solution using a clean pair of forceps before being removed. The remaining extractant solution was then shaken and ca. 1.5 ml of each solution was transferred into a 3 ml capacity auto-sampler tube.  $[\text{NH}_4^+]$  in the extractant solutions were then measured by running them through an Ammonium Flow Injection Analysis (AMFIA) system at the Centre for Ecology and Hydrology, Edinburgh. The analysis was calibrated using three standard solutions of  $\text{NH}_4^+$  (0.1, 1 and 10 ppm), obtained from a 10 ppm stock solution of  $\text{NH}_4\text{Cl}$ . Standard solutions of 0.2, 0.9, 2 and 9 ppm  $\text{NH}_4^+$  were included in each AMFIA run for quality assessment.

### **2.5.4 Calculating Atmospheric $\text{NH}_3$ Concentrations**

The ALPHA samplers operate on the principle of gas diffusion from the atmosphere along an absorbing medium of defined dimensions according to Fick's Law. The theoretical uptake of air by a sampler,  $V$  ( $\text{m}^3$ ) is a function of the length,  $L$  (m) and cross sectional area,  $A$  ( $\text{m}^2$ ) of the stationary air layer

within the sampler and can be calculated provided that the diffusion coefficient  $D$  ( $\text{m}^2 \text{s}^{-1}$ ) of  $\text{NH}_3$  is known. The diffusion coefficient used in the calculation was  $2.09 \times 10^{-5} \text{ m}^2 \text{s}^{-1}$ , as this was the constant used in the CEH NAMN calculations. Using the exposure time,  $t$  (s) of each acid-coated filter paper the effective volume of air sampled by each ALPHA sampler was calculated thus (Eq. 2.2):

$$V = DA t / L \quad [\text{Eq. 2.2}]$$

The  $\text{NH}_3$  concentration,  $\bullet$  ( $\mu\text{g m}^{-3}$ ), for each acid-coated filter paper was then calculated from the extractant  $[\text{NH}_4^+]$  recorded by the AMFIA system from the filter papers of both exposed,  $m_e$  ( $\mu\text{g}$ ) and the control (blank) acid-coated filter papers,  $m_b$  ( $\mu\text{g}$ ). This was done as described below (Eq. 2.3) where  $v$  represents the total volume of extractant solution (3 ml), and (17/18) represents the conversion factor of  $[\text{NH}_4^+]$  into  $[\text{NH}_3]$  based upon the atomic weights of the two compounds.

$$\bullet = \left[ \frac{(m_b - m_e)v}{V} \right] (17/18) \quad [\text{Eq. 2.3}]$$

To obtain monthly  $[\text{NH}_3]$  concentrations for each site the mean of the three acid-coated filter paper replicates received from each site was calculated. A quality control check was then run where replicates that deviated by  $\bullet$  15% from the mean value were excluded. Where exclusion of an acid-coated filter

paper was necessary, the mean for that site was recalculated based upon the remaining replicates.

## **2.6 Modelled Environmental Variables**

Measured concentrations of environmental chemical variables were not available for all the surveyed sites. To compensate for this, estimated concentration and deposition data were obtained from modelled datasets.

Wet ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and NSS  $\text{SO}_4^{2-}$ ), dry ( $\text{NH}_3$ ,  $\text{HNO}_3$ ,  $\text{NO}_2$  and  $\text{SO}_2$ ) and total (rainfall acidity and base cations as obtained from  $\text{Ca}^{2+}$  measurements) deposition values were acquired at the 5 km scale from the Fine Resolution Atmospheric Multi-pollutant Exchange (FRAME) model system (Fournier *et al.*, 2005; Dore *et al.*, 2007; Hallsworth *et al.*, 2010) for the period 2004-2006 based on downloads from national datasets (DEFRA, 2011; DEFRA, 2010).

Concentration data were obtained courtesy of Tony Dore (CEH, Edinburgh) from the FRAME model system. Mean annual concentrations of compounds of nitrogen, sulphur and base cations in aerosol ( $[\text{NH}_3]$ ,  $[\text{HNO}_3]$ ,  $[\text{NO}_2]$ ,  $[\text{SO}_2]$ ) and precipitation ( $[\text{Ca}^{2+}]$ ,  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ) were acquired for analysis at the 1 km and 5 km scales from the FRAME model. The finer scaled 1 km model versions were used to obtain  $[\text{NH}_3]$  and  $[\text{NO}_2]$  data to reduce error due to the local variability in these two compounds (Sutton *et al.*, 1998; Cape *et al.*, 2004; Hallsworth *et al.*, 2010). Also, both  $\text{NH}_3$  and  $\text{NO}_2$  have been observed to have a similar influence on lichen community composition (Davies

*et al.*, 2007; Larsen *et al.*, 2007; Gadsdon *et al.*, 2010). This further highlights the importance of obtaining accurate estimations of these two compounds.

## **2.7 Preliminary Analysis of Environmental Variables**

### **2.7.1 Correlation Analysis of Modelled and Measured Concentration Data for Regional-Scale Survey Sites**

The reliability of the modelled FRAME data were evaluated against the measured data held for each survey site prior to its use as a substitute for the missing measured concentration data values. This was undertaken by examining the goodness of fit between modelled and measured values of each variable using Pearson's Product Moment Correlation Co-efficient (PPMCC). All PPMCC analyses were undertaken following square root transformation of datasets, due to non-normal distributions. Because of limitations in the modelled data available, annual monthly mean concentrations of the measured data were taken for the year 2006 for base cations ( $[\text{Na}^+]$ ,  $[\text{Ca}^{2+}]$  &  $[\text{Mg}^{2+}]$ ) and 2007 for N & S compounds ( $[\text{NH}_3]$ ,  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$ ,  $[\text{HNO}_3]$ ,  $[\text{NO}_2]$ ,  $[\text{SO}_2]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ). The fact that two single years were used raises the question of how historically typical the data used were at each site. An attempt to address this issue was made using the limited measurement data held for the survey sites (Section 2.7.2).

Concentrations of  $\text{NH}_3$  were assessed at 19 out of 28 of the sites as monitoring at the remaining 9 sites was undertaken during 2009/10. Measured values were only available at 10 of the 28 regional sites for  $[\text{NH}_4^+]$ , and 8 of the 28 sites for  $[\text{NO}_3^-]$ ,  $[\text{HNO}_3]$ ,  $[\text{SO}_2]$ , NSS  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ,  $[\text{Na}^+]$ ,  $[\text{Ca}^{2+}]$  and  $[\text{Mg}^{2+}]$ . No

measured data were available for assessment of modelled [NO<sub>2</sub>] data. An assumption was made that the limited datasets would increase the risk of false significant correlations between the modelled and measured variables. Consequently, when assessing the relationship between the two datasets, a high relationship threshold value was subjectively established (PPMCC;  $r \bullet 0.850$ ).

### **2.7.2 Assessment of Measured Data**

In an attempt to understand if the monitoring datasets being used were temporally representative for each site, Kruskal-Wallis analyses of variance were undertaken for each measured variable within each survey site where the appropriate monitoring was undertaken. Kruskal-Wallis was selected over one-way ANOVA due to the non-normal distributions of many of the datasets and difficulties in normalising some sets by various transformation techniques. Where a Kruskal-Wallis analysis indicated a significant difference ( $P \bullet 0.05$ ) in concentrations of a given variable, *post-hoc* Man-Whitney U-tests were performed. If no significant differences were observed between concentration values for the years used in the analysis and the other monitored years in the Mann-Whitney analysis, the measured data used in the assessment of the modelled data were deemed to be typical for the site. Any significant differences ( $P \bullet 0.05$ ) observed were investigated.

### **2.7.3 Assessment of Correlations between Variables**

Once the compatibility of the modelled data was reviewed, it was used to gap-fill the environmental datasets where measured data were missing for sites visited in the survey. The final stage of the environmental assessment was then



undertaken by running PPMCC analyses between environmental variables. This was undertaken to identify strong correlations between variables that would cloud interpretation of the ordination analysis (Section 3.3.5). All concentration data were initially square root transformed due to non-normal distributions. A combination of square root,  $\log_{10}$  and Box-Cox transformations were undertaken on the deposition datasets to obtain normality. Where strong correlations were observed, consideration was given to the omission of one or more of the co-variables from the ordination analyses undertaken in the statistical analysis of biological data (Section 2.13.1). Box-Cox transformations were undertaken in Minitab v.16 based on equation 2.4 below:

$$y' = \begin{cases} (y^{\bullet} - 1)^{\bullet} & \text{where } \bullet > 0 \\ \log y & \text{where } \bullet = 0 \end{cases} \quad [\text{Eq. 2.4}]$$

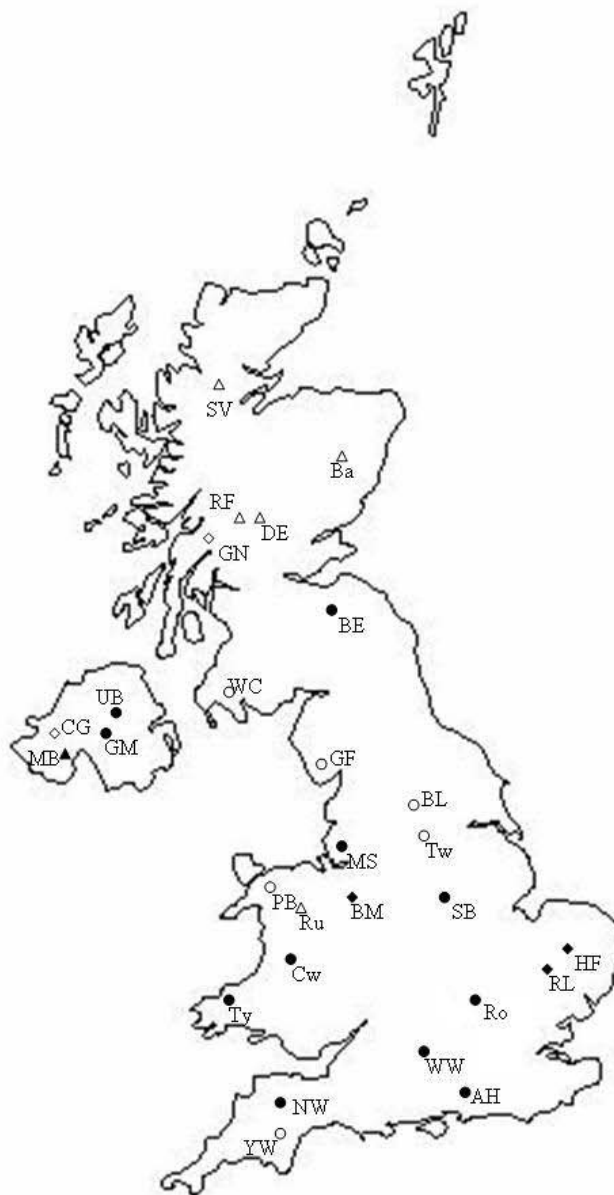
## 2.8 Biological Survey

The selection of trees for surveying followed the methods as described in European guidelines for mapping lichen diversity (Asta *et al.*, 2002), with the exception that trees with noticeable bryophyte cover were not excluded from the survey. This was a result of the requirement to record bryophytes in the survey as a contractual consideration of the project. This was the only major exception, however, and trees with signs of other vegetative growth on or around the trunks were excluded from the survey (e.g. *Hedera helix*, *Ilex aquifolium* and *Rubus fruticosus* agg.).

Healthy, straight-boled trees with a gbh • 50 cm growing in exposed (i.e. not heavily shaded) locations were selected for the epiphytic surveys. Preference was given to trees with low, exposed branches to enable the survey of branches and trunks on the same trees. Where this was not possible, branches from nearby trees of the same species were surveyed. The degree of lean of each surveyed tree was assessed, using a Sylva Expedition 15<sup>®</sup> compass with clinometer, by measuring the angle of inclination/declination of each trunk aspect at the four cardinal compass points.

To reduce the influence of variables associated with phorophyte species, selected trees were narrowed down to four native UK species: *Betula pendula*, *B. pubescens*, *Quercus petraea*, *Q. robur* and their hybrids. The species of each tree surveyed was recorded, before they were grouped together at the generic level (i.e. *Betula* and *Quercus*) for analytical purposes. These trees were chosen because of their native status, wide distribution throughout the UK, acidic bark, and deciduous habit. The criterion of acid bark was an important one, as a previous assessment of lichen responses to [NH<sub>3</sub>] has shown that tree species with a sub-neutral bark pH naturally produce lower bio-monitoring scores to those with acidic bark (Wolseley *et al.*, 2005<sup>a</sup>). This is likely a result of the influence of substrata pH on lichen community composition, which naturally favours lichen species currently identified as being nitrophytic. Nevertheless, it should be noted that the two tree genera differ from each other in a number of physicochemical ways that influence epiphytic lichen communities. Examples include variations in betulin and tannin levels, bark surface pH, lifespan and

levels of bark exfoliation (Atkinson, 1992; James *et al.*, 1977; Jones, 1959; Mitchell, 1974).



**Figure 2.3** Distribution of the UK-wide sites used in the regional-scale survey. Epiphytes were surveyed on two genera of phorophyte at 28 sites. *Quercus* (•) was surveyed at 17 sites, *Betula* (◦) at six and both tree genera (Δ) at five locations. Sites were selected to cover a range of  $[\text{NH}_3]$  that fell above (black) and below (white) the UNECE  $[\text{NH}_3]$  critical level for sensitive epiphyte species of  $1 \mu\text{g m}^{-3}$  based on the finalised dataset (Table 3.4). See Table 2.1 for explanation of site abbreviations.

By limiting the epiphytic surveys to these two genera the study aimed to reduce the influence of phorophyte species on the variability of epiphytic species composition, whilst simultaneously enabling a comparative assessment of the influence that different phorophyte species had upon epiphytic species composition, and subsequently, the bio-monitoring scoring system. *Quercus* alone was surveyed at 17 sites, *Betula* alone at six and both *Quercus* and *Betula* at five sites (Figure 2.3). At sites where both tree genera were surveyed, five individuals of the one genera and three of the other were surveyed because of time constraints in the field.

### **2.8.1 Tree Distance from Monitoring Station**

Trees were sampled over distances of • 1 km from the NH<sub>3</sub> monitoring station at each survey site. Care was taken in all instances to exclude trees situated in areas with point sources of NH<sub>3</sub> emission (e.g. cattle sheds, pig pens, etc.) located between the NH<sub>3</sub> monitoring station and the tree being surveyed.

Distance of trees from monitoring stations (D) were calculated using basic Euclidean geometry (Eq. 2.5), based upon the Ordnance Survey Grid Reference System, using the four Ordnance Survey reference points of surveyed tree easting (T<sub>E</sub>), surveyed tree northing (T<sub>N</sub>), monitoring station easting (S<sub>E</sub>) and monitoring station northing (S<sub>N</sub>).

$$D = \{ \bullet [(T_E - S_E)^2 + (T_N - S_N)^2] \}^{1/2} \quad [\text{Eq. 2.5}]$$

OS co-ordinates for NAMN and AGANet monitoring stations were obtained from the available monitoring network records (DEFRA, 2010). Only a six digit grid reference was available from the NAMN records, which meant distance calculations possessed an error rate of  $\pm 100$  m. The co-ordinates for the nine newly installed  $\text{NH}_3$  monitoring stations and the surveyed trees were obtained *in situ* using a Garmin eTrex<sup>®</sup> Summit personal navigator GPS (Garmin International Inc. 1200 E. 151<sup>st</sup> St., Clathe KS66062. USA). The WGS84 co-ordinates from the GPS were then transformed into OS co-ordinates using a conversion program (Movable Types, 2010).

## **2.8.2 Tree Age and Bark Topography**

Tree girth was recorded at a height of 1.5 m, together with bark topography to provide proxies of tree age and microclimatic variability. Bark topography for each tree was estimated following Ellis & Coppins (2007). Four horizontal transects were drawn running perpendicular across the trunks of each of the four ladder transects undertaken on each tree aspect. The width (fw) and depth (fd) of furrows was measured, together with the length of the two ridges (r) either side of each furrow (Figure 2.4). From this, the length of the furrow habit (fL) was calculated (Eq. 2.6).

$$fL = [\bullet (fw/2)^2 + (fd)^2] 2 \quad \text{[Eq. 2.6]}$$



**Figure 2.4** Technique for recording trunk bark rugosity. The roughness of tree bark was calculated using the measurements of furrow depth (fd), furrow width (fw) and widths of the ridges ( $r_1$  and  $r_2$ ) either side of each furrow.

Bark topography (Bt) was then estimated, based upon the log-quotient of ridge versus furrow habit (Eq. 2.7). The result of using this calculation was that the lowest Bt scores indicated the roughest bark surface.

$$Bt = \log[(r_1 + r_2)/fL] \quad [\text{Eq. 2.7}]$$

## 2.9 Trunk Epiphyte Survey

Epiphytic surveys on the trunks followed the method described by Scheidegger *et al.* (2002). A 16 x 62 cm ladder transect consisting of five 10 x 10 cm quadrats was placed on the four aspects of the trunk relating to each of the four main cardinal compass points (Figure 2.5). Ladder quadrats were placed on each trunk aspect with the baseline of the lowermost quadrat at 1 m above ground level. This was done to reduce the chance of surveying areas

contaminated with abnormally high levels of nitrogenous compounds as a result of issues such as animal marking. Epiphytic species abundance was measured at each survey site by recording the presence of each species identified in every quadrat. Specimens that could not be identified in the field were collected, air-dried, and stored in paper envelopes for identification in the laboratory at a later date (Section 2.11).



**Figure 2.5** Ladder quadrat survey of tree trunks. Photograph courtesy of Ian Leith.

## **2.10 Branch Epiphyte Survey**

The procedure for surveying branch epiphytes followed that of Wolseley *et al.* (2006<sup>a</sup>), as developed from the work of Wolseley & Pryor (1999). Two relatively exposed (i.e. not heavily shaded) branches were selected at 2-5 m above ground level. Branches were cut from the trees to facilitate detailed surveying of both macrolichens and microlichens. Each branch was divided into three age classes: 1-5 years, 6-10 years and 11+ years. For *Quercus*, age classes were obtained by counting the girdle scars along each branch (Figure 2.6) and annual growth was determined by measuring inter-scar distances. In dormant branches, where the terminal bud remained intact, the first year of the branch was taken as the distance between the terminal bud and the first girdle scar. However, once the trees had begun to flush the first year measurements were taken from the first to second girdle scars to ensure exclusion of the current growth year. Because of the difficulty in identifying girdle scars in *Betula*, standardised arbitrary lengths (0-45 cm, 46-75 cm and 76+ cm) were used instead.

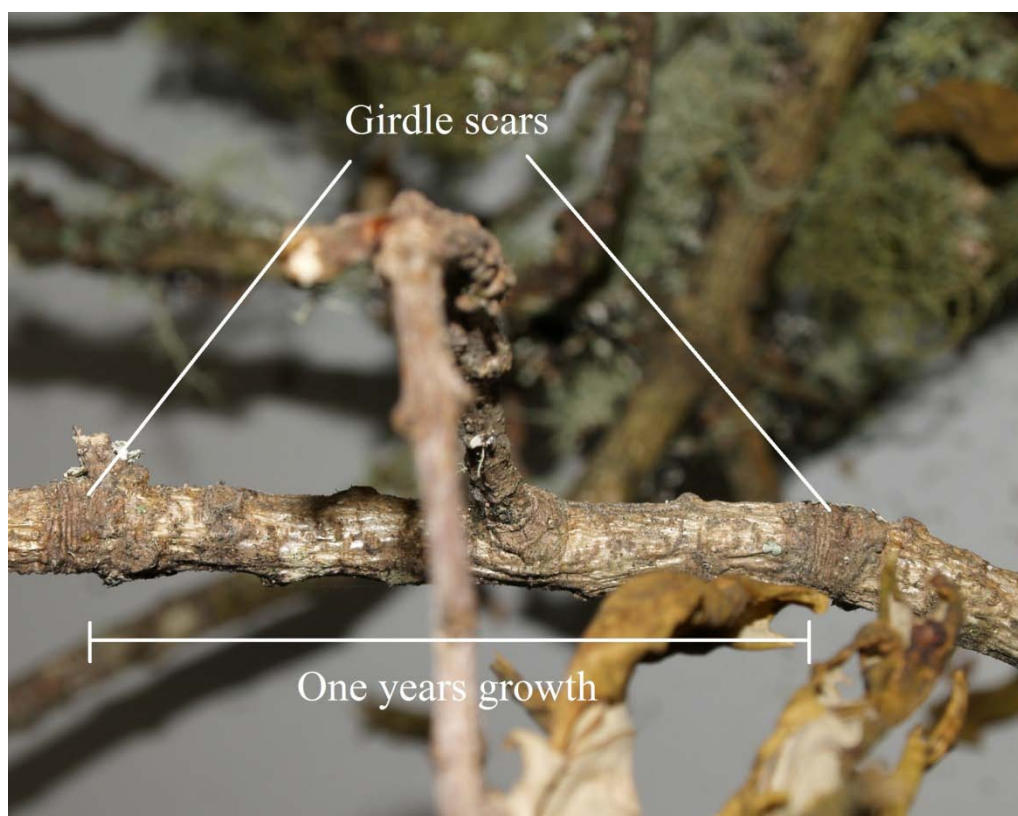
The presence of epiphytic species within each branch age class were recorded, and where necessary, unidentified specimens were collected for laboratory identification in the same manner as that for the trunk epiphyte survey (Section 2.11).

## **2.11 Species Identification**

Species identification during the field surveys was undertaken using Opticron<sup>®</sup> 23 mm x10 and Opticron<sup>®</sup> 18 mm x20 magnification hand lenses for lichens



and bryophytes. Field chemical spot tests using Cl in the form of NaClO from household bleach and K obtained from 10% KOH were also utilised for lichen identifications.



**Figure 2.6** Girdle scars on a *Quercus robur* branch displaying the annual growth of the tree along that section of branch.

More critical laboratory identification was undertaken using a Leica<sup>®</sup> MZ6 dissecting microscope and Schott<sup>®</sup> KL1500LCD lamp, and an Olympus<sup>®</sup> CX41 x40 to x400 high powered light microscope for species that could not be identified in the field. UV light exposure and chemical spot testing with Cl, K and PD (para-phenylenediamine) were also undertaken to assist with lichen identifications where required. The PD was applied in the form of a solution of

the crystals in ethanol. Nomenclature follows that of Smith *et al.* (2009) for lichens, Smith (2004) for Bryophyta, and Paton (1999) for Marchantiophyta.

## **2.12 Bark pH Measurements**

Nitrile gloves were worn at all times during this work. Samples of trunk bark and twigs were collected from all of the trees used in the survey. Any debris (e.g. epiphytes) was removed from each sample where necessary.

Four flat trunk bark samples ca. 2 x 2 cm in size (one from each aspect of the tree), suitable for measuring with a flathead pH electrode were removed from each of the four surveyed trunk aspects of the trees sampled in the survey. Three twig samples measuring 5-7 mm in diameter and ca. 10 cm long were cut from each surveyed branch. Twigs and bark samples were air-dried and stored in paper bags for pH analysis in the laboratory. During the pH analyses, calibration of the pH meter was undertaken with 4.01 and 7.01 buffer solutions when required.

### **2.12.1 Trunk Bark pH Measurements**

Measurements of bark pH were taken using a modified version of the procedure described by Schmidt *et al.* (2001). Bark samples were wetted with 25 mM KCl, and left for 5 minutes. Bark surface pH was then measured for using a HANNAH<sup>®</sup> HI 9026 pH/mV/°C meter and flathead electrode with a small piece of silicon tubing attached to the electrode tip. The tubing extended 1.5 mm above the surface of the 7 mm diameter flathead surface electrode and enabled the placement of 0.24 ml of 25 mM KCl into the cup formed, which

provided a liquid medium that sufficiently covered the electrode sensors. The wetted bark specimens were then placed over the tubing and pH value noted when a stable reading was obtained after ca. 2-5 minutes. Mean pH values from each of the four replicates were then calculated and used as a representation of trunk bark pH values for each individual tree.

### **2.12.2 Branch pH Measurements**

Twig pH analysis followed the methodology used by Wolseley *et al.*, (2006<sup>a</sup>), as adapted from Kermit & Gauslaa, (2001). Prior to measuring twig pH, all the twigs were cut to ca. 6 cm lengths and the exposed cut twig ends were sealed with paraffin wax before each twig was soaked in 0.25 mM KCl in sealed tubes to prevent CO<sub>2</sub> ingress. Each sample was left to soak for 1 h with intermittent shaking. Further to established methods (Wolseley *et al.*, 2006<sup>a</sup>; Kermit & Gauslaa, 2001), an attempt to standardise the volume of KCl added relative to the surface area of each twig was made. This was done based on a 20 mm<sup>2</sup> : 1 ml twig area to KCl volume ratio. Under the assumption that the twigs were cylindrical, twig surface area (A) was calculated based upon the width (W) and length (L) of each twig (Eq. 2.8).

$$A = \pi WL \quad [\text{Eq. 2.8}]$$

Twigs were removed after one hour and the pH of the solution measured with a Mettler Toledo<sup>®</sup> MP 220 pH meter with Inlab 427 solid state combination puncture gel electrode. The mean value of the three twig sample replicates was used as an estimate of twig substratum pH for each branch.

## **2.13 Statistical Analysis of Biological Data**

An assessment of variation of epiphytic species richness was undertaken on the trunks and branches of surveyed trees. Epiphytes were separated into three broad groups: bryophytes, macrolichens (species with a fruiticose, foliose or squamulose growth forms) and microlichens (leprose, crustose or endophloedal species). Analysis was undertaken using a one-way ANOVA with Tukey HSD. Observations on patterns of SR of the three epiphytic groups were also undertaken along the three age classes of branches. This was undertaken using Box-Cox transformed data in a one-way ANOVA analysis with Tukey HSD for macro- and microlichens. Comparisons of the two lichen forms with bryophytes along the three branch age classes was undertaken using Mann-Whitney U-tests due to the non-normal distribution of bryophytes along the branch age classes. Differences in lengths of the two branch age classes 1-5 years and 6-10 years were also compared using one-way ANOVA. Data for this analysis was  $\log_{10}$  transformed to obtain normality.

### **2.13.1 Ordination Analysis**

Separate ordination analyses were run on the epiphytes growing on the trunks and branches of trees surveyed in the survey. Non-linear Multi-Dimensional Scaling (NMDS) was undertaken on the arcsine transformed proportional data of epiphytes identified on the trunks and branches at each site. The proportional data were derived from the recorded frequency of occurrence of each species on each tree, either in the quadrats or along the branch sections surveyed, expressed as a proportion of the total number of quadrats or branch sections per tree trunk or branch. A select number of climatic and

environmental variables, which were identified from the procedures described in Chapter 3, were entered into the matrix for NMDS analysis.

NMDS analyses with a Monte Carlo algorithm and Sørensen distance measurements were run using PC-Ord<sup>TM</sup> v.6 (Mather, 1976; Kruskal 1964<sup>a</sup>; Kruskal 1964<sup>b</sup>). Preliminary step down ordinations from six dimensions with a stress test were run for both trunk and branch data to determine dimensionality. A total of 500 iterations with random starting points were run for the data. Once established, the dimensionality was set for the trunk and branch data and five ordinations undertaken with 250 iterations of the real data for both. Mantel tests were undertaken between the ordinations to evaluate the reliability of the ordination patterns. Plots of the NMDS ordinations were made, and the percentage of variance explained in the distance matrix was evaluated using a Sørensen distance measure for both original and ordination space.

### **2.13.2 Analysis to Identify Indicator Species**

Indicator Species Analysis (ISA) was undertaken in PC-Ord<sup>TM</sup> v.6. Indicator values (*IV*) representing the percentage of perfect indication of each species were obtained from the product of the proportional abundance and proportional frequency of each species following the method of Dufrêne & Legendre (1997). Analysis was only undertaken on species that were present in at least three survey sites. Proportional data for species on each surveyed trunk and branch were established as the main (response) matrix and [NH<sub>3</sub>] as the second (explanatory) matrix. All data in the response matrix were arcsine transformed prior to analysis. The explanatory matrix was determined by using a [NH<sub>3</sub>]

threshold of  $1 \mu\text{g m}^{-3}$ , a value that reflected the current United Nations Economic Commission for Europe critical level for epiphytes and sensitive habitats (Cape *et al.*, 2009). A Monte Carlo permutation test was undertaken with 5000 randomised runs to identify epiphytes that were significantly classed as either nitrophobic or nitrophilic. Once indicator species were identified, their percentage frequency of occurrence on *Quercus* (Q%fo) and *Betula* (B%fo) trees across the 28 survey sites was calculated. From these two values a phorophyte-based frequency of occurrence ratio (Q:B) was obtained to assess the relative abundance of each indicator species on the two phorophytes studied in the survey (Eq. 2.9).

$$\text{Q:B} = \text{Q\%fo} / \text{B\%fo} \quad [\text{Eq. 2.9}]$$

A shortlist was then produced for indicator species to be considered for use in the NH<sub>3</sub> bio-monitoring scheme. These species were identified based upon the following considerations.

1. A significant ( $P < 0.05$ ) result in the indicator species analysis.
2. Good UK-wide distribution.
3. Non-specificity to a particular phorophyte.
4. Ease of identification.
5. An equal number of nitrophobic and nitrophilic indicators.
6. Found growing on both trunks and branches.
7. Cross-referencing of identified indicator species against established literature and knowledge.

### 2.13.3 Assessment of $L_{AN}$ Scoring System

A revised form of the  $L_{AN}$  scoring system was evaluated using data acquired in the survey for the shortlisted indicator species. Unlike the previous abundance-based  $L_{AN}$  technique (Wolseley *et al.*, 2005<sup>a</sup>), this was undertaken using a simple frequency-based approach.

A frequency-based approach was attempted for three main reasons. First, an abundance-based technique was not feasible given the epiphytic sampling technique used for either the trunks (Section 2.9) or branches (Section 2.10). Second, a frequency-based approach would produce more standardised results to an abundance-based technique, as inconsistencies resulting from the individual subjectivity of species area coverage recorded by surveyors would be negated. Finally, a frequency-based technique would remove the underlying bias that an abundance-based technique possess towards lichens with a spreading, foliose growth habit over fruticose and crustose species.

Four variations of the Frequency-based  $L_{AN}$  ( $FL_{AN}$ ) scores for trunks and branches were tested against the  $\log_{10}$  values of measured  $[NH_3]$  at each site using PPMCC analysis. In order to compare scores for trunks and branches the trunk data were manipulated to provide a 0-3 frequency score equivalent to the branch frequency scores, worked examples of which can be found in Appendix K. This was achieved by assessing three of the four most lichen-covered tree aspects (east, south and west) and by clumping the data within the five quadrats surveyed on each aspect. Consideration was given to factoring down the trunk scores to the same scale as that of the branches by means of simple calculus.

However, a rapid and straightforward method of surveying the trunks had to be considered for the bio-monitoring scheme when it was implemented into a field guide for use by conservation agencies and government bodies. The four FL<sub>AN</sub> scoring schemes formulated and assessed were as follows:

**a) Non-accumulative and Un-factored FL<sub>AN</sub> (NUFL<sub>AN</sub>)**

This was the most basic approach and focussed on clumping indicator frequency counts at the ecological class level (i.e. nitrophobes and nitrophiles) rather than scoring each individual indicator species separately. The total number of quadrats/branch sections that nitrophilic indicators (N) were present in on each tree was subtracted from the total of nitrophobic species (A). The site NUFL<sub>AN</sub> score was then determined by dividing the total A – N scores for each surveyed tree by the number of tree surveyed (**n**) By calculating a score based purely on nitrophobe and nitrophile indicator frequencies on trunks and branches (Eq. 2.10) the influence of SR and diversity was removed. This approach produced a range of scores from -3 to +3 per site, based on the three branch section/trunk aspect approach used.

$$\text{NUFL}_{\text{AN}} = \frac{\bullet (A - N)}{n} \quad [\text{Eq. 2.10}]$$

**b) Non-accumulative, Factored FL<sub>AN</sub> (NFFL<sub>AN</sub>)**

The NFFL<sub>AN</sub> introduced SR-weighting by multiplying the N and A values for each tree surveyed by the number of different nitrophilic species (n<sub>i</sub>)



and nitrophobic species ( $a_i$ ) identified on each individual tree respectively, before subtracting the derived nitrophilic score from the nitrophobic one. Final site  $NFFL_{AN}$  scores were then observed in the same manner as the  $NUFL_{AN}$ , from the mean score of each tree surveyed (Eq. 2.11). This provided a range of scores from -27 to +27 per site.

$$NFFL_{AN} = \frac{\sum (Aa_i - Nn_i)}{n} \quad [\text{Eq. 2.11}]$$

**c) Accumulative, Un-factored  $FL_{AN}$  ( $AUFL_{AN}$ )**

Unlike the previous two scoring methods, the  $AUFL_{AN}$  and  $AFFL_{AN}$  were more detailed and scored sites based on the recording of indicators at the species level. On each tree, the sum frequency of each individual nitrophilic species recorded ( $N_i$ ) was subtracted from that of the sum frequency of each individual nitrophobic species recorded ( $A_i$ ). Site  $AULF_{AN}$  scores were then determined from the mean score of each tree surveyed at the location (Eq. 2.12). As with the  $NFFL_{AN}$ , this approach also produced a scoring range of -27 to +27 per site.

$$AUFL_{AN} = \frac{\sum (A_i - N_i)}{n} \quad [\text{Eq. 2.12}]$$

**d) Accumulative and Factored  $FL_{AN}$  ( $AFFL_{AN}$ )**

This was the most heavily weighted of the four techniques. Scores for each tree were obtained by multiplying the sum frequency of each individual

nitrophilic species ( $N_i$ ) by the number of different nitrophilic indicators recorded on each tree ( $n_i$ ). This value was then subtracted from the product of the sum frequencies of nitrophobic species ( $A_i$ ) and number of nitrophobic species recorded on the tree ( $a_i$ ). Final values were then determined in the same manner as previous scoring systems (Eq. 2.13). This method results in a maximum potential scoring range of -243 to +243 per site.

$$AFFL_{AN} = \frac{\bullet (A_i a_i - N_i n_i)}{n} \quad [\text{Eq. 2.13}]$$

#### 2.13.4 Evaluation of Indicator Species

To test the robustness of the new shortlist of indicator species, scores of the best fitting  $FL_{AN}$  were compared against those calculated based upon the established indicator species lists (Appendix C) used in previous studies into atmospheric N pollution (OPAL, 2011; Wolseley *et al.*, 2009).

The results of the new shortlist of indicator species were predicted to produce a better all round fit and accuracy when compared to the OPAL indicator species list (OPAL, 2011) and that used in the study by Wolesely *et al.* (2009) for three main reasons. Firstly, the new shortlist is based on a UK specific list of indicators; secondly, it includes microlichens; and finally, modifications to the taxonomic level of indicator species have been made (i.e. *Parmelia* and *Hypogymnia* indicators are now described at the specific rather than generic level).

### **2.13.5 Phorophyte Calibration**

Trunk and branch FL<sub>AN</sub> scores on *Betula* and *Quercus* trees were plotted separately against measured NH<sub>3</sub> concentrations to evaluate differences in scores between the two phorophyte genera. The goodness of fit between FL<sub>AN</sub> scores derived from each of the two tree genera was obtained using a PPMCC. An attempt to calibrate scores between the two phorophyte genera was then made by plotting trunk and branch FL<sub>AN</sub> scores for *Quercus* and *Betula* against each other at the five sites where both genera were surveyed.

### **2.13.6 Environmental and Climatic Relationships with FL<sub>AN</sub> Scores**

Scores of the best fitting FL<sub>AN</sub> were plotted against other environmental and climatic variables to identify relationships between them. Any observed correlations were reviewed before finding a best fit model based upon the strongest correlating variables.

## **Chapter 3. Regional-Scale Environmental Data**

### **3.1 Introduction**

Data for a total of 29 environmental variables were obtained for analysis; 11 of these were climatic and 18 were components of atmospheric chemistry. Two issues required resolution for these datasets. Firstly, the concentration data were comprised of a mixture of Fine Resolution Atmospheric Multi-pollutant Exchange model (FRAME) modelled data and measured data, acquired either personally during 2009-2010 or through the National Ammonia Monitoring Network (NAMN) and Acid Gas and Aerosol Network (AGANet) over a variable range of years. Consequently a review of these datasets was required to identify any major inconsistencies in these recorded values relating to the reliability of the FRAME modelled values and any anomalous temporal variation in the datasets. Secondly, the large number of variables would risk over-fitting the subsequent ordination analyses. An attempt was made to reduce the number of variables used in ordinations by a combination of selective omission and targeted exclusion based upon the identification of strongly co-correlated variables.

Assessment of air chemistry variables was undertaken using Pearson's Product Moment Correlation Co-efficient (PPMCC) analyses, following appropriate transformation of some datasets where required. All normality tests for the analysed datasets were undertaken using an Anderson-Darling test. Climate variables were analysed non-parametrically using Spearman's Rank Correlation (SRC) analysis due to non-normal data distributions.

Before the PPMCC analyses could be undertaken on the air chemistry variables the reliability of the modelled data used to supplement missing measured data required assessment. This was done by evaluating the goodness of fit between the modelled data and the mean annual monthly measured values for the corresponding year, where available, using a PMCC. To increase the reliability of this assessment the measured air chemistry variables at each site were also compared temporally to other recording years to evaluate their likely reliability as a suitable representative measurement.

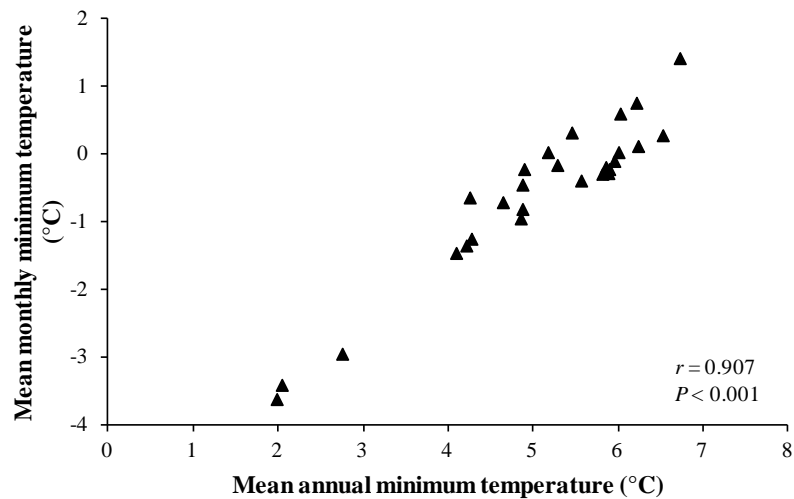
## **3.2 Climate Data**

A 30 year dataset for temperature and precipitation at all 28 sites was utilised. Mean annual temperatures across all sites ranged from 4.8°C ( $\pm 0.2^\circ\text{C}$ ) at Strath Vaich to 10.1°C ( $\pm 0.3^\circ\text{C}$ ) at Wytham Wood (Table 3.1). Mean annual precipitation varied from 615 mm yr<sup>-1</sup> ( $\pm 4.7$ ) at Hinderclay Fen to 2624 mm yr<sup>-1</sup> ( $\pm 17.4$ ) at Plas-y-Brenin. Mean annual rain days > 1 mm ranged from 117 ( $\pm 2.2$ ) at Sutton Bonnington to 227 ( $\pm 3.0$ ) at Strath Vaich (Table 3.1).

### **3.2.1 Correlation Analyses of Climate Data**

Relationships between climatic variables and the oceanicity index (OI) were analysed using SRC. This exercise did not constitute autocorrelation because two of the three climatic components used in the derivation of the OI were excluded. The exception was mean minimum monthly temperature ( $T_{\min}$ ), which was shown to be poorly correlated with the OI (Table 3.2). The results showed that all temperature-based variables were negatively correlated, and precipitation-based variables positively correlated, with the OI (Table 3.2).

This observed pattern could be explained by the structure of the formula used to calculate OI (Section 2.3.1). The OI scores, as a representative of climatic variation across sites, were observed to correlate well ( $r_s = 0.70$ ) with eight of the ten climate variables. The two exceptions were mean annual minimum temperature ( $T_{\min}$ ) ( $r_s = -0.593$ ) and  $T_{\min}$  ( $r_s = -0.335$ ). A very strong positive correlation was observed for these two temperature variables using SRC analysis (Figure 3.1), indicating that, under the assumption of co-correlation of these two factors, one of them could be excluded from the Non-linear Multi-dimensional Scaling (NMDS) analysis.



**Figure 3.1** Relationship between mean annual minimum temperature and mean monthly minimum temperature derived from a 30 year dataset. Spearman's Rank Correlation analyses of the two variables produced a significant correlation.

**Table 3.1** Mean annual and monthly climatic data for the 28 regional-scale survey sites based on a 30-year dataset from 1977-2006.

Regional-Scale Survey Site	Site Code	Annual Precipitation (mm y <sup>-1</sup> )	Annual Rain Days >1mm	Annual Rain Days >10mm	Annual Temperature	Annual Min. Temperature	Annual Max. Temperature	Annual Growing Degree Days >5°C	Monthly Min. Temperature*	Monthly Max. Temperature*	Monthly Growing Degree Days > 5°C*	Oceanicity Index†
Alice Holt	AH	798	120.1	21.8	10.1	5.9	14.3	2027	-0.2	22.9	169	5.20
Banchory	Ba	831	137.3	23.7	7.9	4.1	11.6	1364	-1.5	19.0	114	6.71
Bretton Lakes	BL	705	123.9	19.4	9.6	6.0	13.2	1883	0.0	21.3	157	5.84
Brown Moss	BM	732	136.5	17.8	9.4	5.6	13.3	1860	-0.4	21.2	155	6.33
Bush Estate	BE	1006	155.6	27.5	7.5	4.2	10.8	1261	-1.4	18.0	105	8.03
Correl Glen	CG	1585	220.1	56.9	7.7	4.3	11.2	1257	-0.6	17.9	105	11.86
Cwmystwyth	Cw	1663	192.0	63.8	8.2	4.9	11.5	1467	-0.8	18.7	122	9.85
Dunalastair Estate	DE	1240	177.4	36.7	6.5	2.8	10.1	1039	-3.0	17.7	87	8.59
Glen Nant	GN	2521	209.0	82.4	8.1	4.6	11.6	1416	-0.7	18.4	118	10.92
Glenmore Wood	GM	1016	183.6	32.8	8.4	4.9	11.9	1467	-0.2	18.7	122	9.69
Grizedale Forest	GF	1824	189.2	69.4	8.1	4.3	11.9	1450	-1.3	19.2	120	9.27
Hinderclay Fen	HF	615	118.1	13.1	9.9	5.9	14.0	2004	-0.2	22.7	167	5.16
Mere Sands Wood	MS	888	145.6	23.4	9.7	6.2	13.2	1936	0.1	20.6	161	7.13
Moninea Bog	MB	1239	176.5	30.2	9.3	5.5	13.1	1752	0.3	20.0	146	8.97
North Wyke	NW	1074	154.9	34.1	9.7	6.2	13.1	1854	0.8	20.4	154	7.88
Plas y Brenin	PB	2624	194.1	78.5	8.8	5.3	12.3	1596	-0.2	19.5	133	9.88
Rannoch Forest	RF	1708	196.3	53.2	5.3	2.0	8.7	812	-3.4	16.2	68	10.03
Redgrave & Lopham Fen	RL	633	120.5	14.3	9.8	5.8	13.8	1969	-0.3	22.5	164	5.29
Rothamsted	Ro	709	119.8	18.0	9.8	5.9	13.7	1940	-0.3	22.5	162	5.27
Ruabon	Ru	1010	164.6	32.1	8.2	4.9	11.6	1466	-1.0	19.1	122	8.20
Strath Vaich	SV	1814	226.7	61.8	4.8	2.0	7.7	732	-3.6	14.5	61	12.53
Sutton Bonnington	SB	628	117.1	14.8	9.9	6.0	13.8	1967	-0.1	22.2	164	5.26
Twentywellsick Wood	Tw	888	136.5	24.1	9.8	6.3	13.2	1930	0.2	21.5	161	6.43
Tycanol Wood	Ty	1586	158.9	45.3	9.9	6.7	13.1	1902	1.4	19.6	158	8.74
Upper Ballinderry River	UB	1100	178.3	31.6	8.8	5.2	12.3	1570	0.0	18.7	131	9.53
Wood of Cree	WC	1480	178.2	52.8	8.7	4.9	12.6	1588	-0.5	19.6	132	8.87
Wytham Wood	WW	666	120.1	17.9	10.1	6.5	13.7	2061	0.3	22.4	172	5.44
Yarner Wood	YW	1512	159.6	50.3	9.2	6.0	12.4	1732	0.6	19.8	144	8.31

\* (• 30 12 monthly values)/360

† See Section 2.2.1

**Table 3.2** Spearman's Rank Correlation analysis of climatic variables. The Oceanicity Index (OI) was evaluated against two precipitation (PPN) variables, including number of rain days (RD) per year, and six variables based on temperature (T) including growing degree days (GDD) > 5°C, given as both monthly and annual means. Values for variables not correlated with the OI at the  $r_s > 0.70$  level are indicated in bold.

	PPN	RD	T	T <sub>min</sub>	T <sub>max</sub>	T <sub>min</sub>	GDD	GDD
	(annual)	>10mm	(annual)	(annual)	(annual)	(monthly)	(annual)	(monthly)
OI*	0.913	0.902	-0.753	<b>-0.596</b>	-0.830	<b>-0.341</b>	-0.793	-0.793

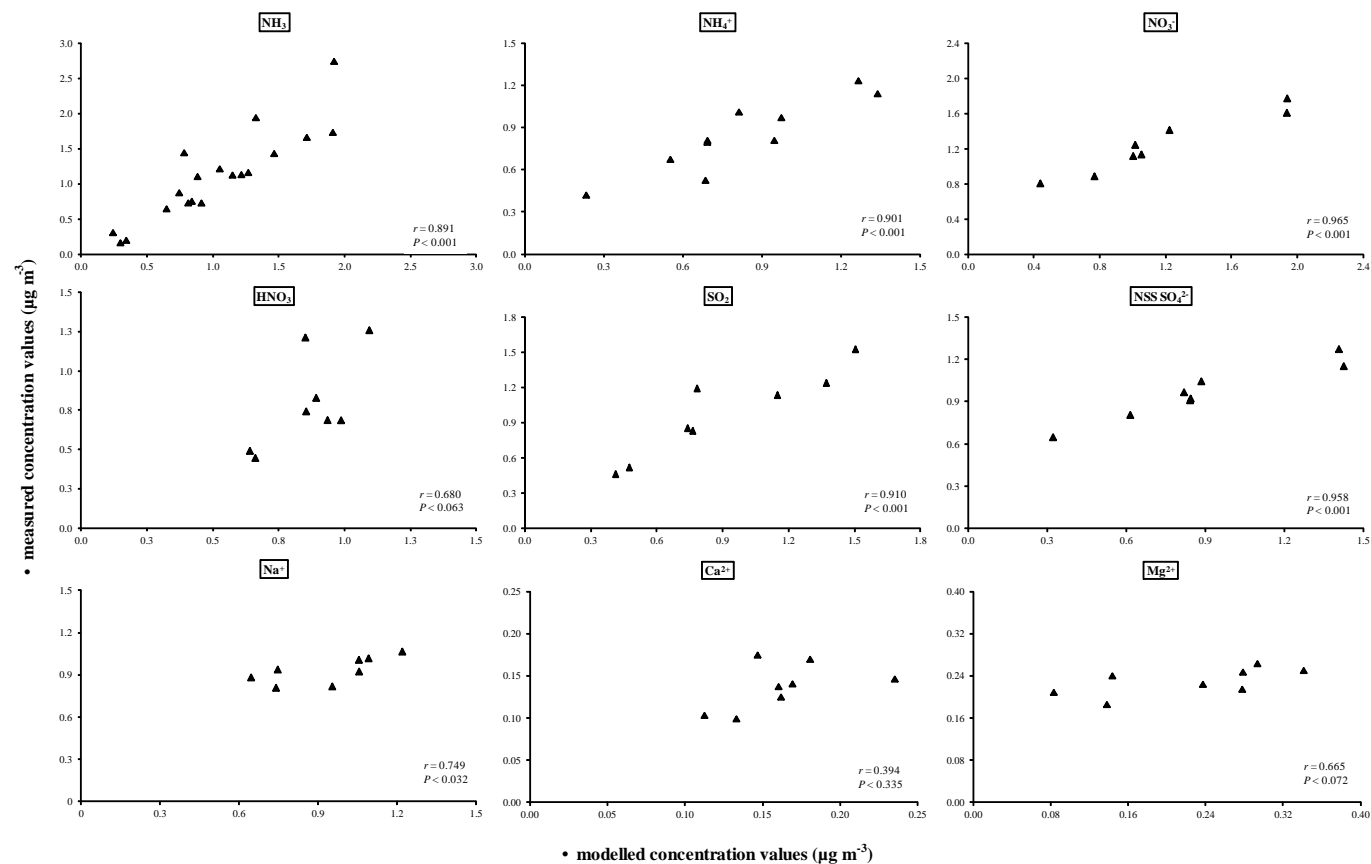
\* See Section 2.2.1

### **3.3 Atmospheric Chemistry Data**

#### **3.3.1. Comparisons between Modelled and Measured Concentrations of Gases and Aerosols**

The goodness of fit between the modelled chemical concentration data and the limited measured concentrations available was examined using a PPMCC. Strong correlations were found between  $[\text{NH}_3]$ ,  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$ ,  $[\text{SO}_2]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$  (Figure 3.2). Some individual disparities between variables were observed at some sites. Notable anomalies were in  $\text{NH}_3$  values at Cwmystwyth where the modelled and measured mean annual monthly concentrations were  $0.61 \mu\text{g m}^{-3}$  and  $2.10 \mu\text{g m}^{-3}$  respectively. This discrepancy could arise if the survey location was located near to the point sources used to calculate the  $\text{NH}_3$  concentrations for that specific modelled 1 km square. Two further possible underestimations of  $[\text{NH}_3]$  were observed at Sutton Bonnington (modelled =  $1.75 \mu\text{g m}^{-3}$ , measured =  $3.80 \mu\text{g m}^{-3}$ ) and Hinderclay Fen (modelled =  $3.67 \mu\text{g m}^{-3}$ , measured =  $7.56 \mu\text{g m}^{-3}$ ). A further anomaly was the poor correlation (PPMCC;  $n = 8$ ;  $r = 0.0680$ ;  $P = 0.063$ ) between modelled and measured data for  $[\text{HNO}_3]$  and [base cations] (Figure 3.2). These disparities might have been due to technical problems in the measuring methods of  $\text{HNO}_3$  and  $\text{Ca}^{2+}$  (J.N. Cape, Pers. Comm., 2011).





**Figure 3.2** Pearson's Product Moment Correlation Coefficient analysis of modelled FRAME and measured environmental data for 2006 ([base cations]) and 2007 ([N] and [S] compounds).

In view of the above relationships, it was decided that subsequent ordination and correlation analyses (Chapters 4 and 5) would utilize measured values of N and S concentrations and modelled values of base cations and  $\text{HNO}_3$  concentrations. The strong relationships between measured and modelled N and S data facilitated relatively precise prediction of missing values in measured datasets. In addition supplementary measurements of  $\text{NH}_3$  were made at many sites as part of the present project. In contrast, the relationships between measured and modelled values of base cations and  $\text{HNO}_3$  concentrations were considered too poor to predict with an acceptable degree of accuracy values at sites where measurements were not made and, further, it was not considered feasible to make supplementary measurements for these chemical species

### **3.3.2           Assesment of the Reliability of Measured Atmospheric Chemistry Data for 2007**

The concentration data used in later ordination and correlation analyses were selected for a single year: all N and S species for 2007; and base cations for 2006. In the case of N and S, it was considered important to assess the typicalness of the 2007 data in comparison with values for other years in order to identify possible anomalous values.

Sites at which there was significant year to year variation in measured N or S concentrations were identified by Kruskal-Wallis analyses (Table 3.3). A more detailed assessment of the data from these sites was then undertaken using Mann-Whitney U-Tests between the 2007 data and those of other years. A

non-parametric approach was used instead of a One-Way ANOVA because of the non-normal distribution of the datasets.

Three types of temporal pattern could be identified among the measured datasets. The most marked temporal pattern observed among sites was a steady decline in concentrations. This was clearly evident for [SO<sub>2</sub>] levels during the nine year monitoring periods at both Bush Estate and Sutton Bonnington. In both instances the 2007 value was significantly (Mann-Whitney U-Test;  $P < 0.05$ ) lower than those for several of the previous monitoring years (Figure 3.4). Significant differences (Mann-Whitney U-Test;  $P < 0.05$ ) were also observed between median [SO<sub>2</sub>] values for 2007 and other years at Rothamsted and Plas y Brenin; the former of which displayed a similar pattern to the Bush Estate and Sutton Bonnington. Only three years worth of data was available for Plas y Brenin and this was not deemed sufficient for conclusions to be drawn.

Annual median [NH<sub>4</sub><sup>+</sup>] values also showed a pattern of smooth declines, and 2007 values were identified as being significantly (Mann-Whitney U-Test;  $P < 0.05$ ) lower than those of earlier years at three sites (Bush Estate, Mere Sands Wood and Sutton Bonnington) this pattern was displayed clearly at two of the sites, but data from 2001-2003 was unavailable for the third site (Bush Estate), making confirmation of the trend more difficult. Given the continued decline in measured median [SO<sub>2</sub>] and [NH<sub>4</sub><sup>+</sup>] values the 2007 data were accepted as being part of a natural trend for these variables at all sites.

The second most frequent pattern of variation was a smooth trend suggesting a temporal pattern. In some cases, a further decline in concentrations occurred in later years implying a potential smooth decline reflecting the trend in  $[\text{SO}_2]$  data, e.g. that of annual median  $[\text{SO}_4^{2-}]_{\text{NSS}}$  at the Bush Estate (Figure 3.5a) and Sutton Bonnington (Figure 3.5b).

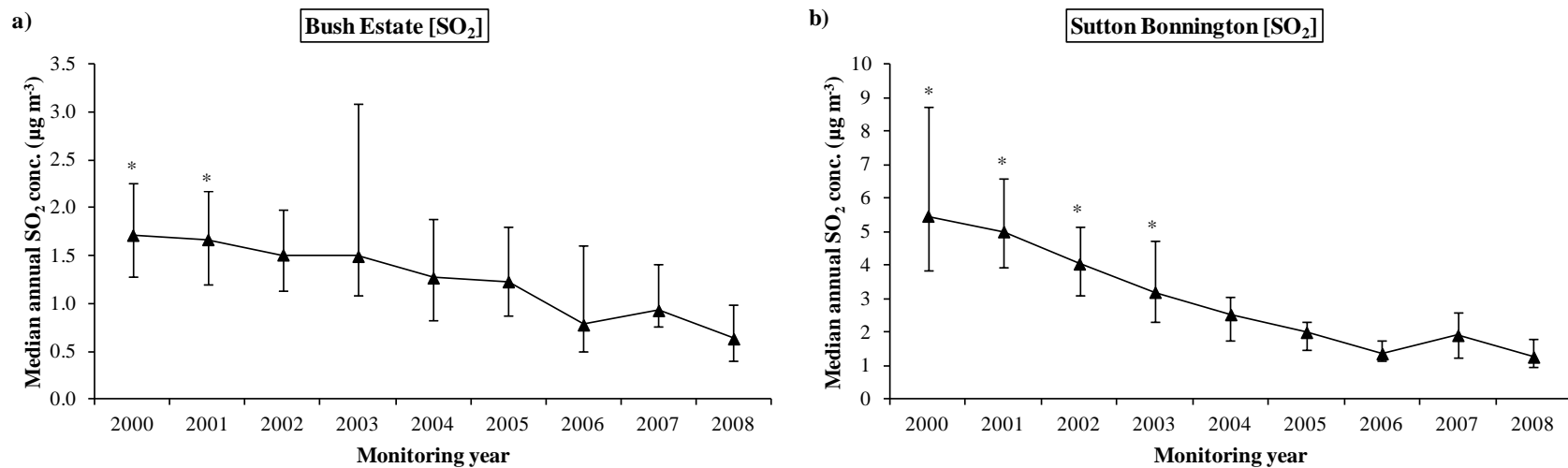
Smooth trends were particularly marked for  $\text{NH}_3$  data, e.g. at the Bush Estate (Figure 3.5c) and Wytham Wood (Figure 3.5d). In both of these examples the annual median  $[\text{NH}_3]$  value for 2007 were significantly (Mann-Whitney U-Test;  $P \bullet 0.05$ ) different from years with the lowest concentrations during the monitoring period. In these instances the 2007 values were considered representative of the sites since they were components of a consistent trend in inter-annual variation.

Finally, data for several sites showed apparently random inter-annual variation, or “noise”. For seven of the eight remaining Kruskal-Wallis site/variable analyses (Table 3.3), 2007 values were either not found to be significantly different (Mann-Whitney;  $P \bullet 0.05$ ) to any other years (Correl Glen  $[\text{SO}_2]$  and Yarner Wood  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ), or only significantly different (Mann-Whitney;  $P \bullet 0.05$ ) to one other year.

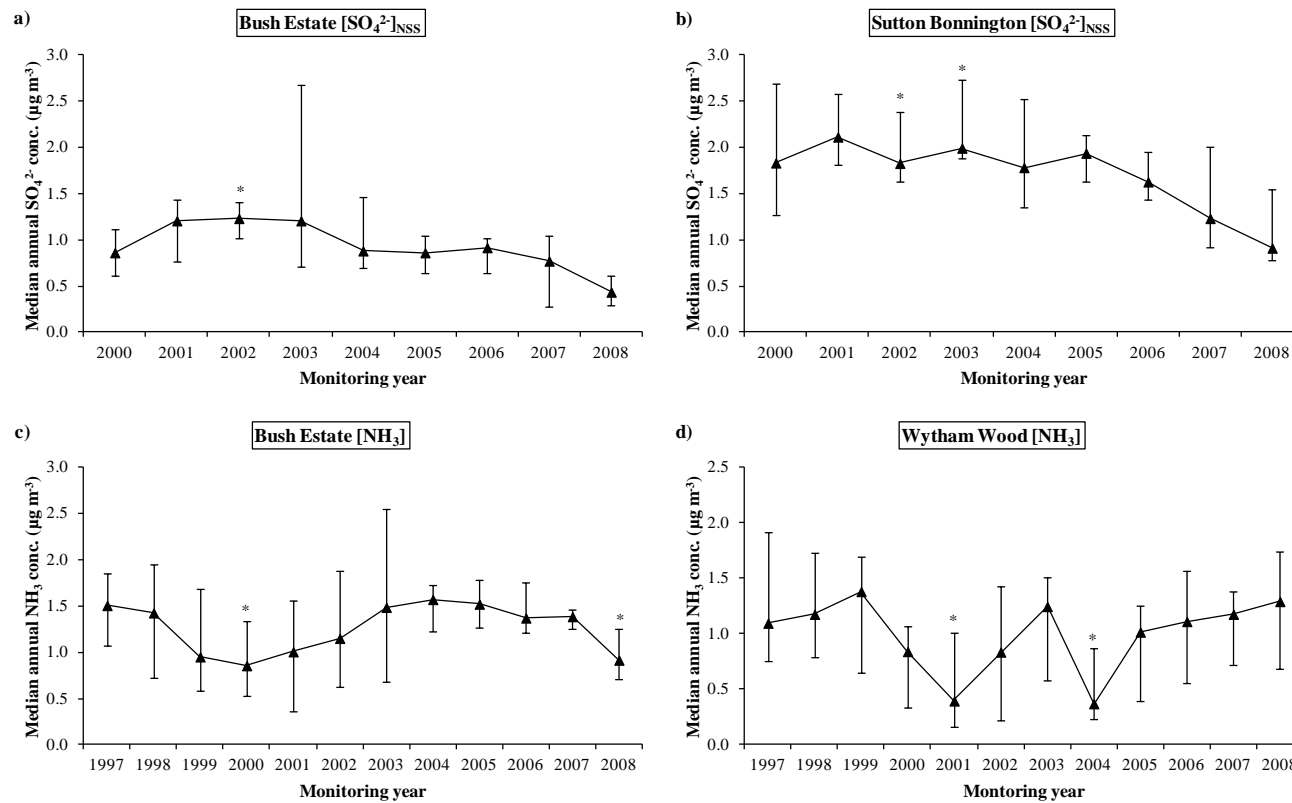
The remaining dataset (Brown Moss) showed marked year to year variation or “noise” (Figure 3.6). The median  $[\text{NH}_3]$  for 2007 at this site was perhaps the least reliable chemical value in the dataset since the median annual  $\text{NH}_3$  value for this year was below typical historical concentrations.

**Table 3.3** Kruskal-Wallis analyses of the temporal atmospheric [N] and [S] variation within sites. Significant ( $P < 0.05$ ) differences between median annual concentrations were observed at sites over designated monitoring periods (bold type). Italicised  $P$  values are adjusted for ties.

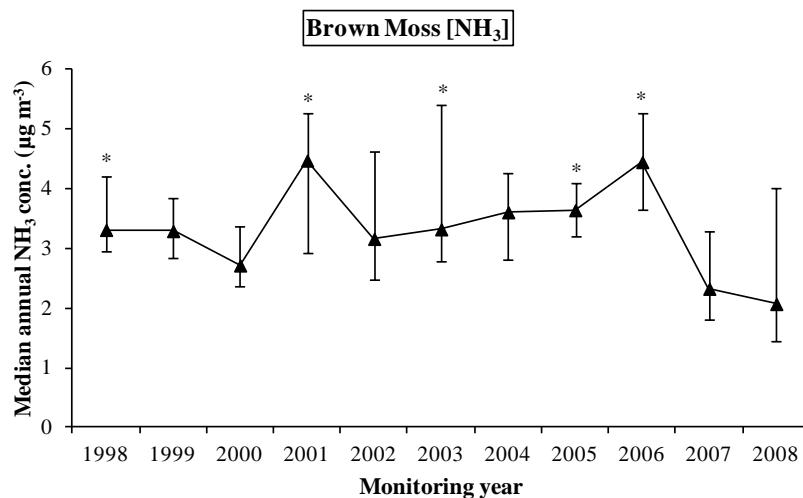
Site Name	Monitoring Period	[NH <sub>3</sub> ] ( $\mu\text{g m}^{-3}$ )	[NH <sub>4</sub> <sup>+</sup> ] ( $\mu\text{g m}^{-3}$ )	[NO <sub>3</sub> ] ( $\mu\text{g m}^{-3}$ )	[SO <sub>2</sub> ] ( $\mu\text{g m}^{-3}$ )	[SO <sub>4</sub> <sup>2-</sup> ] <sub>NSS</sub> ( $\mu\text{g m}^{-3}$ )
Alice Holt	1998-2008	0.056	-	-	-	-
Brown Moss	1998-2008	<b>0.005</b>	-	-	-	-
Bush Estate	1997-2008	<b>0.037</b>	<b>0.001</b>	0.235	<b>0.002</b>	<i>0.001</i>
Correl Glen NR	1997-2008	<i>0.196</i>	0.701	0.802	<b>0.035</b>	0.068
Cymystwyth	1997-2008	0.266	0.136	0.140	<b>0.018</b>	<b>0.020</b>
Dunalastair Estate	2001-2008	<i>0.116</i>	-	-	-	-
Grizedale Forest	2001-2008	0.330	-	-	-	-
Hinderclay Fen	2007-2010	0.741	-	-	-	-
Mere Sands Wood	1997-2008	<b>0.046</b>	<b>0.032</b>	-	-	-
North Wyke	1997-2008	<i>0.231</i>	0.342	-	-	-
Plas y Brenin	2006-2008	<i>0.458</i>	<i>0.563</i>	0.454	<b>0.021</b>	0.482
Rannoch Forest	2001-2008	<i>0.246</i>	-	-	-	-
Redgrave & Lopham Fen	1997-2008	<i>0.151</i>	-	-	-	-
Rothamsted	1997-2008	<i>0.610</i>	0.086	0.191	<b>0.000</b>	<b>0.006</b>
Ruabon	1997-2008	<b>0.000</b>	-	-	-	-
Strath Vaich Dam	1997-2008	<i>0.471</i>	<i>0.812</i>	<i>0.661</i>	<i>0.243</i>	<b>0.046</b>
Sutton Bonnington	1997-2008	0.576	<b>0.002</b>	0.209	<b>0.000</b>	<b>0.001</b>
Wytham Wood	1997-2008	<b>0.023</b>	-	-	-	-
Yarner Wood	1997-2008	0.996	0.326	0.293	<b>0.008</b>	<b>0.029</b>



**Figure 3.4** Temporal variation in annual median measured SO<sub>2</sub> concentrations at (a) the Bush Estate and (b) Sutton Bonnington where there were significant (Mann-Whitney;  $P < 0.05$ ) differences between the 2007 value and other years (\*). Error bars represent inter-quartile ranges.



**Figure 3.5** Temporal variation in annual median measured NSS  $\text{SO}_4^{2-}$  (Figures a,b) and  $\text{NH}_3$  (Figures c,d) concentrations at sites where significant (Mann-Whitney;  $P < 0.05$ ) differences between the 2007 data and other years existed (\*). Error bars represent inter-quartile ranges.



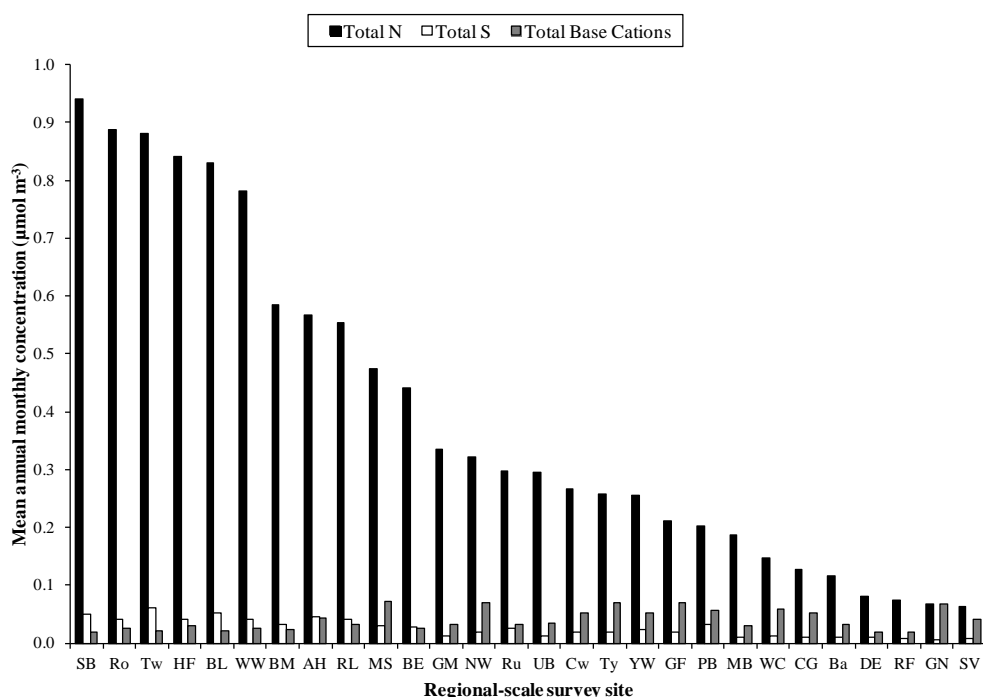
**Figure 3.6** Observed inter-annual variation of median annual NH<sub>3</sub> concentrations as derived from the NAMN for Brown Moss, where significant (Mann-Whitney;  $P < 0.05$ ) differences between the 2007 data and other years were seen to exist (\*). Error bars represent inter-quartile ranges.

### 3.3.3 Atmospheric Chemistry Variables Selected: Concentration Data

Following the initial analysis of modelled and measured concentration data (Sections 3.2.1 and 3.2.2) the modelled monthly mean values for [NH<sub>4</sub><sup>+</sup>], [NO<sub>3</sub><sup>-</sup>], [SO<sub>2</sub>], and [SO<sub>4</sub><sup>2-</sup>]<sub>NSS</sub> were accepted as substitutes at sites where measured data were not available for 2007. Modelled data alone were used for [HNO<sub>3</sub>] and [base cations] due to potential errors in measurement techniques see (Section 3.2.1), and for [NO<sub>2</sub>] due to a lack of measured data. Despite the relative reliability of modelled [NH<sub>3</sub>] values, a decision was made to use measured data for all sites, creating disparities in monitoring years: values for 2009/10 were used for nine of the 10 sites, based upon the monitoring stations erected as part of this project (Section 2.5), and values for 2007 for Hinderclay Fen and the remaining 18 sites that were part of the NAMN (Table 2.1).



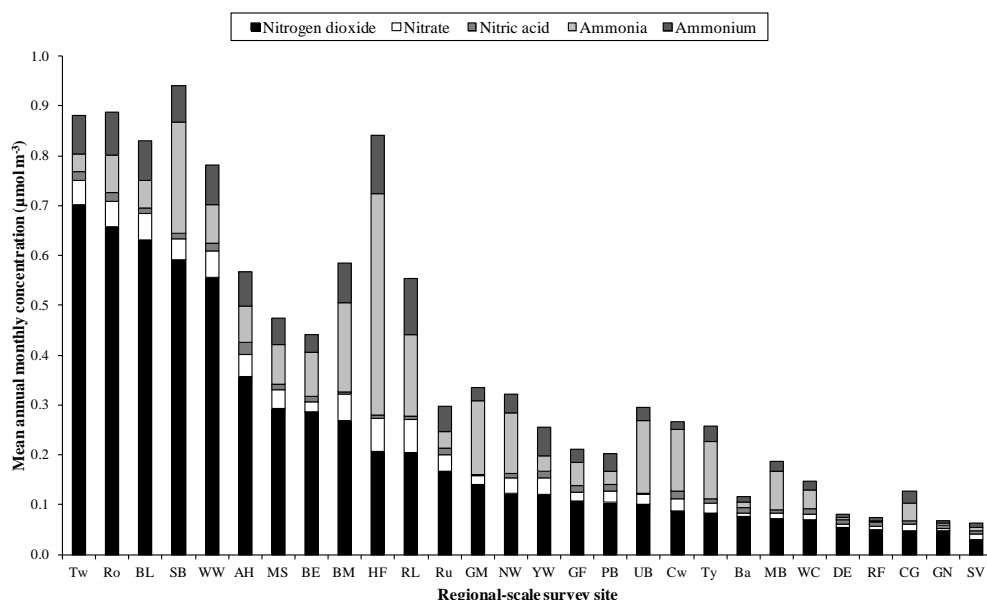
Molar concentrations of total [N] far exceed those of total [S] and [base cations], values for which are  $< 0.1 \text{ mol m}^{-3}$  (Figure 3.7). An assessment of the nitrogenous compounds showed that molar concentrations of gaseous  $[\text{NO}_2]$  were the greatest, comprising  $> 50\%$  of the total [N] at most sites. Gaseous  $[\text{NH}_3]$  was the second most abundant compound, followed by aerosol  $[\text{NH}_4^+]$  and  $[\text{NO}_3^-]$  (Figure 3.8).



**Figure 3.7** Mean annual molar concentrations of total nitrogen ( $[\text{NH}_3] + [\text{NH}_4^+] + [\text{NO}_3^-] + [\text{HNO}_3] + [\text{NO}_2]$ ), sulphur ( $[\text{SO}_2] + [\text{SO}_4^{2-}]_{\text{NSS}}$ ) and base cations ( $[\text{Na}^+] + [\text{Ca}^{2+}] + [\text{Mg}^{2+}]$ ) for the 28 UK regional-scale survey sites ranked in order of decreasing molar [N].

Mean annual  $[\text{NH}_3]$  values were below the UNECE critical level of  $1 \mu\text{g m}^{-3}$  at 13 sites, and in four of these,  $< 0.1 \mu\text{g m}^{-3}$  (Dunalastair Estate, Glen Nant, Rannoch Forest and Strath Vaich). This critical level was exceeded at the remaining 15 sites, with  $[\text{NH}_3]$  values between  $1\text{--}3 \mu\text{g m}^{-3}$  at most of them; but

higher concentrations were recorded at three (Brown Moss, Hinderclay Fen and Sutton Bonnington) (Table 3.4).



**Figure 3.8** Annual monthly mean molar concentrations of nitrogenous compounds for the 28 UK regional-scale survey sites ranked in order of decreasing molar  $[\text{NO}_2]$ .

The three most  $[\text{NH}_3]$  polluted sites were located in areas where the 30 year mean annual precipitation was  $< 750 \text{ mm yr}^{-1}$ . Conversely, three of the four cleanest sites were in regions with a mean annual precipitation  $> 1700 \text{ mm yr}^{-1}$ . A PPMCC analysis of the relationship between  $[\text{NH}_3]$  and precipitation revealed that  $[\text{NH}_3]$  values were negatively correlated with mean annual precipitation (Figure 3.9).

Total mean annual concentrations of  $[\text{S}]$  and  $[\text{base cations}]$  were very low compared to those of  $[\text{N}]$ . The maximum observed total  $[\text{S}]$  was  $< 5 \mu\text{g m}^{-3}$  and below the  $[\text{SO}_2]$  critical level for cryptogams (WHO, 2011). However, the decline in  $[\text{SO}_2]$  levels at Sutton Bonnington from  $5.92 (\pm 0.31) \mu\text{g m}^{-3}$  in 2000

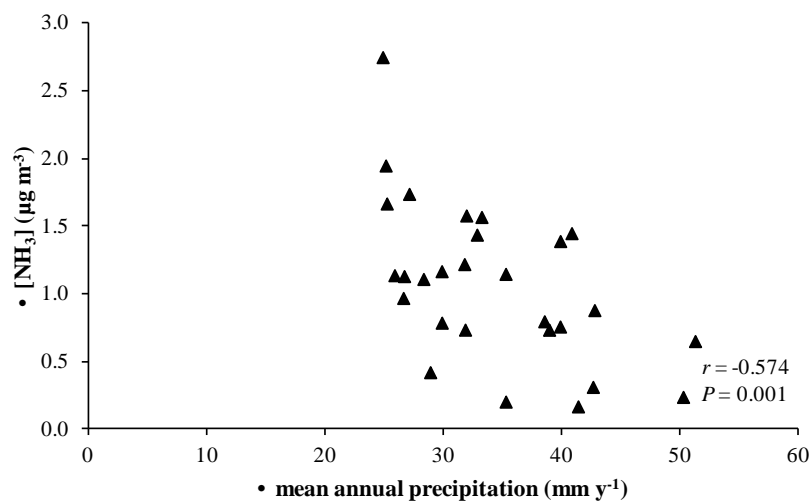
to 1.35 ( $\pm 0.12$ )  $\mu\text{g m}^{-3}$  in 2008 (Section 3.2.2.5) highlights the concern that temporal effects of previously high  $[\text{SO}_2]$  values may still influence lichen community composition at certain sites, and subsequently affect the bio-monitoring scoring system. If this is the case, its influence should, in theory, be restricted to the lichens identified in the trunk surveys (Section 2.9) due to the relative longevity of the substratum compared to that of the surveyed branches (Section 2.10).

**Table 3.4** Environmental variable concentration dataset used to evaluate the 28 regional-scale survey sites in the analyses. Values obtained from FRAME modelled data are italicised. Sites exceeding the UNECE critical level of 1  $\mu\text{g m}^{-3}$  for  $[\text{NH}_3]$  are in bold.

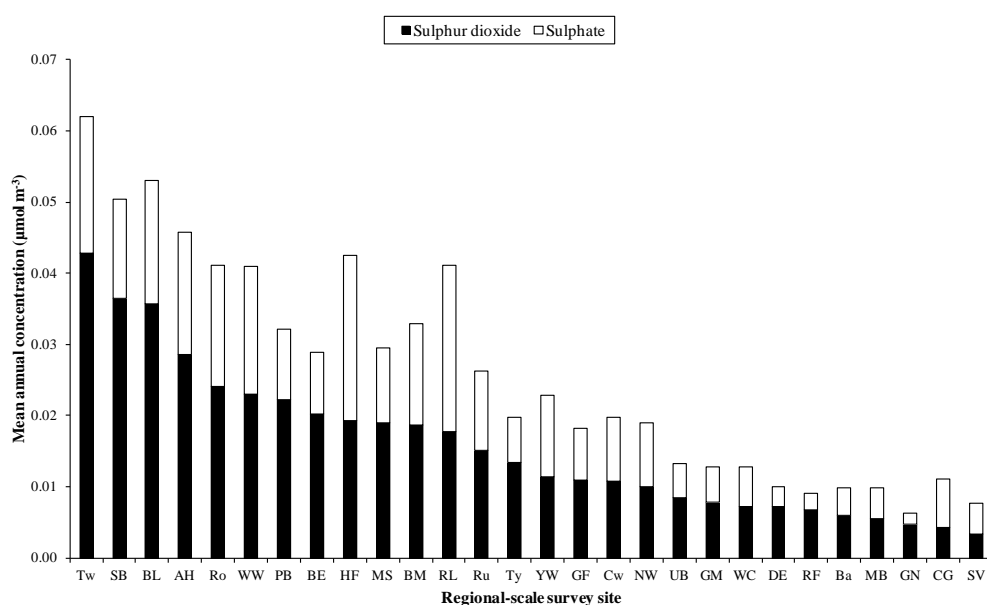
Regional-Scale Survey Site	$[\text{NH}_3]$	$[\text{NH}_4^+]$	$[\text{NO}_3^-]$	$[\text{HNO}_3]$	$[\text{NO}_2]$	$[\text{SO}_2]$	$[\text{SO}_4^{2-}]_{\text{Nss}}$	$[\text{Na}^+]$	$[\text{Ca}^{2+}]$	$[\text{Mg}^{2+}]$
Alice Holt	<b>1.24</b>	<i>1.25</i>	<i>2.81</i>	<i>1.49</i>	<i>16.43</i>	<i>1.84</i>	<i>1.65</i>	<i>0.91</i>	<i>0.04</i>	<i>0.06</i>
Banchory	0.18	<i>0.23</i>	<i>0.52</i>	<i>0.63</i>	<i>3.49</i>	<i>0.38</i>	<i>0.37</i>	<i>0.71</i>	<i>0.01</i>	<i>0.03</i>
Bretton Lakes	0.94	<i>1.43</i>	<i>3.21</i>	<i>0.72</i>	<i>29.07</i>	<i>2.29</i>	<i>1.65</i>	<i>0.47</i>	<i>0.03</i>	<i>0.01</i>
Brown Moss	<b>3.03</b>	<i>1.43</i>	<i>3.29</i>	<i>0.36</i>	<i>12.35</i>	<i>1.20</i>	<i>1.37</i>	<i>0.53</i>	<i>0.01</i>	<i>0.02</i>
Bush Estate	<b>1.49</b>	<i>0.64</i>	<i>1.26</i>	<i>0.73</i>	<i>13.15</i>	<i>1.30</i>	<i>0.83</i>	<i>0.54</i>	<i>0.01</i>	<i>0.02</i>
Correl Glen	0.58	<i>0.46</i>	<i>0.80</i>	<i>0.41</i>	<i>2.26</i>	<i>0.27</i>	<i>0.65</i>	<i>1.11</i>	<i>0.03</i>	<i>0.08</i>
Cwmystwyth	<b>2.10</b>	<i>0.28</i>	<i>1.56</i>	<i>0.97</i>	<i>4.01</i>	<i>0.70</i>	<i>0.86</i>	<i>1.11</i>	<i>0.03</i>	<i>0.08</i>
Dunalastair Estate	0.04	<i>0.15</i>	<i>0.43</i>	<i>0.61</i>	<i>2.52</i>	<i>0.46</i>	<i>0.26</i>	<i>0.43</i>	<i>0.00</i>	<i>0.01</i>
Glen Nant	0.06	<i>0.08</i>	<i>0.25</i>	<i>0.47</i>	<i>2.19</i>	<i>0.30</i>	<i>0.15</i>	<i>1.43</i>	<i>0.04</i>	<i>0.11</i>
Glenmore Wood	<b>2.50</b>	<i>0.50</i>	<i>1.10</i>	<i>0.24</i>	<i>6.46</i>	<i>0.50</i>	<i>0.48</i>	<i>0.71</i>	<i>0.02</i>	<i>0.04</i>
Grizedale Forest	0.78	<i>0.50</i>	<i>1.08</i>	<i>0.94</i>	<i>4.92</i>	<i>0.71</i>	<i>0.69</i>	<i>1.47</i>	<i>0.04</i>	<i>0.12</i>
Hinderclay Fen	<b>7.56</b>	<i>2.10</i>	<i>4.17</i>	<i>0.43</i>	<i>9.51</i>	<i>1.24</i>	<i>2.23</i>	<i>0.68</i>	<i>0.02</i>	<i>0.03</i>
Mere Sands Wood	<b>1.37</b>	<i>0.95</i>	<i>2.27</i>	<i>0.70</i>	<i>13.54</i>	<i>1.21</i>	<i>1.01</i>	<i>1.52</i>	<i>0.05</i>	<i>0.12</i>
Moninea Bog	<b>1.32</b>	<i>0.35</i>	<i>0.71</i>	<i>0.39</i>	<i>3.35</i>	<i>0.36</i>	<i>0.41</i>	<i>0.63</i>	<i>0.02</i>	<i>0.03</i>
North Wyke	<b>2.07</b>	<i>0.66</i>	<i>1.96</i>	<i>0.57</i>	<i>5.66</i>	<i>0.64</i>	<i>0.87</i>	<i>1.47</i>	<i>0.04</i>	<i>0.11</i>
Plas y Brenin	0.43	<i>0.66</i>	<i>1.30</i>	<i>0.87</i>	<i>4.90</i>	<i>1.43</i>	<i>0.94</i>	<i>1.19</i>	<i>0.03</i>	<i>0.09</i>
Rannoch Forest	0.03	<i>0.12</i>	<i>0.37</i>	<i>0.58</i>	<i>2.32</i>	<i>0.43</i>	<i>0.23</i>	<i>0.43</i>	<i>0.00</i>	<i>0.01</i>
Redgrave & Lopham Fen	<b>2.79</b>	<i>2.04</i>	<i>3.99</i>	<i>0.46</i>	<i>9.47</i>	<i>1.14</i>	<i>2.24</i>	<i>0.69</i>	<i>0.02</i>	<i>0.03</i>
Rothamsted	<b>1.28</b>	<i>1.53</i>	<i>3.16</i>	<i>1.19</i>	<i>30.23</i>	<i>1.54</i>	<i>1.63</i>	<i>0.55</i>	<i>0.02</i>	<i>0.02</i>
Ruabon	0.54	<i>0.93</i>	<i>2.08</i>	<i>0.86</i>	<i>7.69</i>	<i>0.97</i>	<i>1.07</i>	<i>0.68</i>	<i>0.03</i>	<i>0.03</i>
Strath Vaich	0.10	<i>0.18</i>	<i>0.66</i>	<i>0.43</i>	<i>1.42</i>	<i>0.22</i>	<i>0.42</i>	<i>1.48</i>	<i>0.06</i>	<i>0.12</i>
Sutton Bonnington	<b>3.80</b>	<i>1.31</i>	<i>2.61</i>	<i>0.72</i>	<i>27.21</i>	<i>2.34</i>	<i>1.33</i>	<i>0.41</i>	<i>0.03</i>	<i>0.01</i>
Twentywellsick Wood	0.62	<i>1.38</i>	<i>3.00</i>	<i>1.02</i>	<i>32.34</i>	<i>2.74</i>	<i>1.85</i>	<i>0.42</i>	<i>0.07</i>	<i>0.01</i>
Tycanol Wood	<b>1.94</b>	<i>0.58</i>	<i>1.27</i>	<i>0.60</i>	<i>3.79</i>	<i>0.86</i>	<i>0.61</i>	<i>2.78</i>	<i>0.08</i>	<i>0.26</i>
Upper Ballinderry River	<b>2.46</b>	<i>0.51</i>	<i>1.17</i>	<i>0.18</i>	<i>4.68</i>	<i>0.55</i>	<i>0.46</i>	<i>0.76</i>	<i>0.03</i>	<i>0.04</i>
Wood of Cree	0.64	<i>0.31</i>	<i>0.62</i>	<i>0.76</i>	<i>3.24</i>	<i>0.47</i>	<i>0.53</i>	<i>1.26</i>	<i>0.03</i>	<i>0.09</i>
Wytham Wood	<b>1.30</b>	<i>1.44</i>	<i>3.26</i>	<i>1.05</i>	<i>25.60</i>	<i>1.47</i>	<i>1.73</i>	<i>0.54</i>	<i>0.02</i>	<i>0.02</i>
Yarner Wood	0.54	<i>1.03</i>	<i>2.01</i>	<i>0.79</i>	<i>5.58</i>	<i>0.73</i>	<i>1.10</i>	<i>1.14</i>	<i>0.02</i>	<i>0.06</i>

Annual mean  $[\text{SO}_2]$  values were greater than those for aerosol  $[\text{SO}_4^{2-}]_{\text{Nss}}$  (Figure 3.10). At most sites, total  $[\text{S}]$  values were lower than those of [base cations] on a molar basis; the exceptions were 10 sites, most of which were

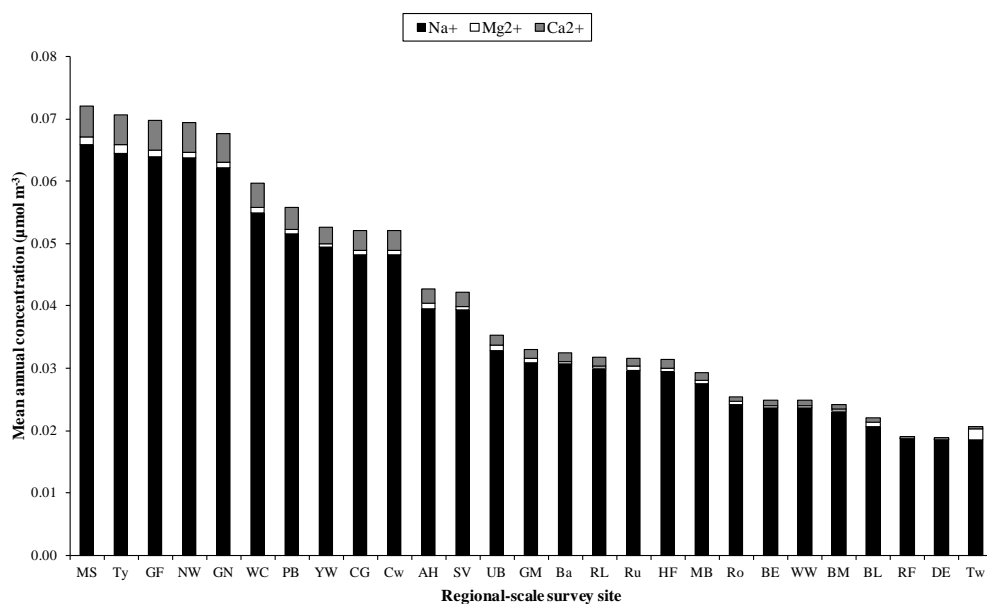
located in the southeast of the UK (Figure 3.7). The overriding dominant component of the [base cation] group was  $[\text{Na}^+]$  (Figure 3.11).



**Figure 3.9** The relationship between mean monthly  $[\text{NH}_3]$  values and mean annual precipitation at the 28 UK regional-scale survey sites. PPMCC analysis showed a significant negative correlation between the two variables.



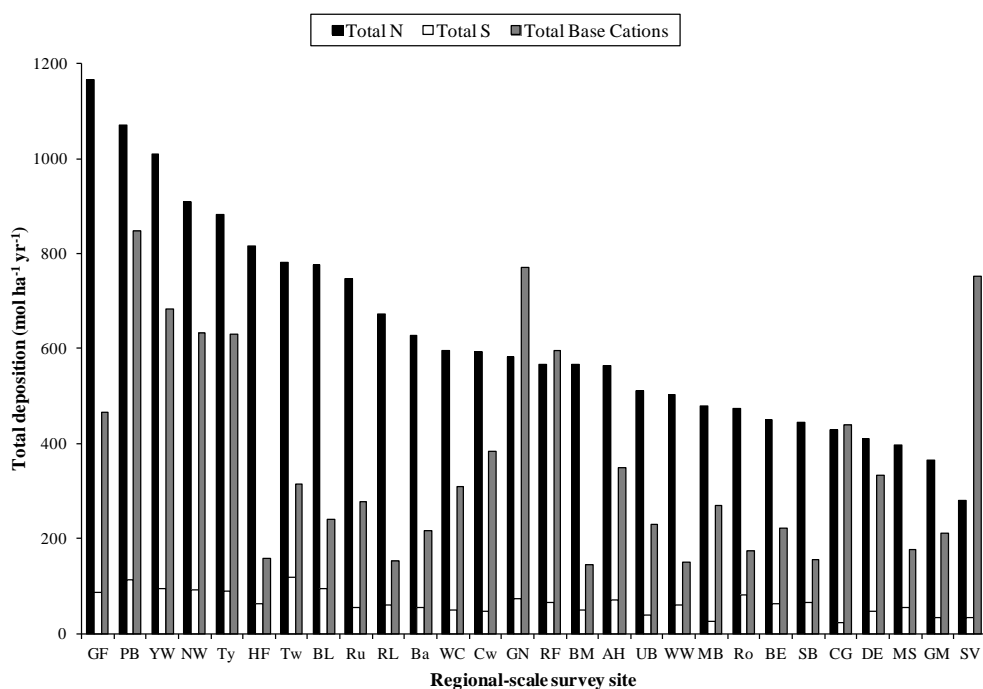
**Figure 3.10** Annual monthly mean concentrations of sulphurous compounds at the 28 UK regional-scale survey sites ranked in order of decreasing  $[\text{SO}_2]$ .



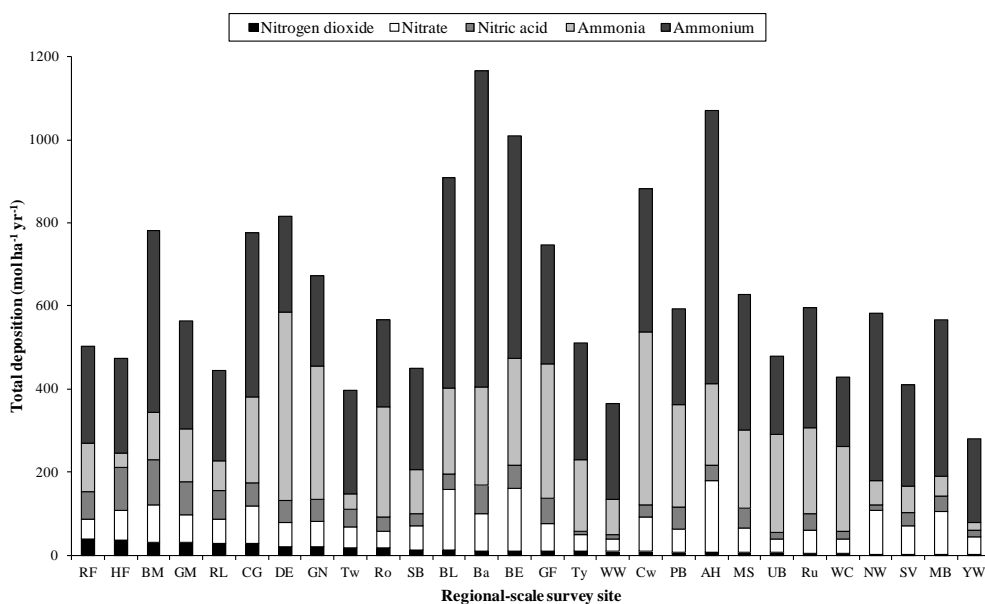
**Figure 3.11** Annual mean concentrations of base cations at the 28 UK regional-scale survey sites ranked in order of decreasing  $[\text{Na}^+]$ .

### 3.3.4 Atmospheric Chemistry Variables Selected: Deposition Data

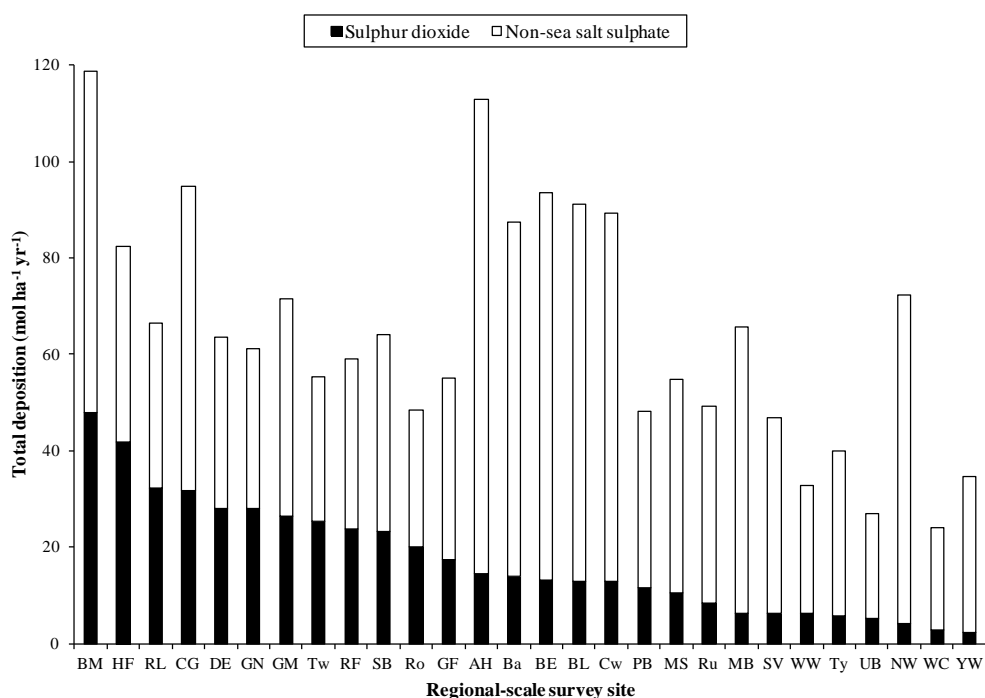
Total wet and dry deposition values for all chemical species were derived from the modelled FRAME dataset for 2006 (DEFRA, 2011; Dore *et al.*, 2007). Nitrogen was typically the most abundant of the three groups of atmospheric chemical species in wet and dry deposition, being considerably greater than S inputs. This reflected a similar species ranking amongst the concentration data. However, the ranking of sites in order of decreasing N deposition differed for deposition and concentration data (Figure 3.12). Of the individual components of deposition ionic species predominate compared to gases (Figures 3.13-3.14). However, one notable exception was the greater number of moles of  $\text{NH}_3$  in deposition compared to  $\text{NO}_3^-$  (Figure 3.13).  $\text{NO}_2$  constituted a relatively small fraction of nitrogen deposition. Deposition of base cations was also much higher than sulphur on a molar basis at all sites, and exceeded total N deposition at three sites in the northwest of the UK (Figure 3.12).



**Figure 3.12** Total annual mean deposition of total nitrogen ( $\text{NH}_3 + \text{NH}_4^+ + \text{NO}_3^- + \text{HNO}_3 + \text{NO}_2$ ), sulphur ( $\text{SO}_2 + \text{NSS SO}_4^{2-}$ ) and base cations ( $\text{Na}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$ ) at the 28 UK regional-scale survey sites ranked in order of decreasing N deposition.



**Figure 3.13** Total annual mean deposition of nitrogenous compounds at the 28 UK regional-scale survey sites ranked in order of decreasing  $\text{NO}_2$  deposition.



**Figure 3.14** Total annual mean deposition of sulphurous compounds at the 28 UK regional-scale survey sites ranked in order of decreasing SO<sub>2</sub> deposition.

### 3.3.5 Selection of Environmental Variables for Use in Ordination Analyses

Use of the large number of environmental variables in subsequent ordination analyses was not practical, and would overfit the model. In an attempt to reduce the number of variables a subjective decision was taken to omit all deposition data from the ordination analyses in the first instance on the assumption that the bio-monitoring scheme being developed was to be based on N concentration in air rather than deposition values. However, the relationship between deposition values and scores from the bio-monitoring scheme was investigated later (Section 5.3.1). Altitude was also omitted from the initial ordination because of restricted sampling in that only sites below 500 m were selected to remove the influence of occult precipitation.

In order to reduce the number of remaining chemical variables, the atmospheric concentration factors were assessed for co-correlation using PPMCC analysis (Table 3.5). All non-normal data were normalised prior to analysis using either square root or Box-Cox transformations. The strongest correlations were between aerosol  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ . These relationships were reflected in a strong correlation between cationic  $[\text{NH}_4^+]$  and anionic ( $[\text{NO}_3^-] + [\text{SO}_4^{2-}]_{\text{NSS}}$ ) on a molar basis (Figure 3.15).

**Table 3.5** Pearson's Product Moment Correlation Coefficient analysis of environmental variable concentrations at the 28 UK sites. Numbers in bold represent significant relationships between variables at the  $P \bullet 0.001$  level

		<i>P</i> -value									
		$[\text{NH}_3]^*$	$[\text{NH}_4^+]^*$	$[\text{NO}_3^-]$	$[\text{HNO}_3]$	$[\text{NO}_2]^\dagger$	$[\text{SO}_2]^*$	$[\text{SO}_4^{2-}]_{\text{NSS}}^*$	$[\text{Na}^+]^\dagger$	$[\text{Ca}^{2+}]^*$	$[\text{Mg}^{2+}]^*$
<i>r</i> value	$[\text{NH}_3]^*$	-	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.385	0.008	0.043	0.001	0.577	0.325	0.840
	$[\text{NH}_4^+]^*$	<b>0.650</b>	-	<b>&lt;0.001</b>	0.138	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.488	0.167	0.242
	$[\text{NO}_3^-]$	<b>0.647</b>	<b>0.967</b>	-	0.127	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.330	0.235	0.171
	$[\text{HNO}_3]$	-0.171	0.287	0.295	-	0.010	0.002	0.023	0.666	0.235	0.995
	$[\text{NO}_2]^\dagger$	0.492	<b>0.846</b>	<b>0.817</b>	0.481	-	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.296	0.197	0.055
	$[\text{SO}_2]^*$	0.386	<b>0.790</b>	<b>0.767</b>	0.553	<b>0.919</b>	-	<b>&lt;0.001</b>	0.278	0.103	0.094
	$[\text{NSS SO}_4^{2-}]^*$	0.585	<b>0.964</b>	<b>0.966</b>	0.428	<b>0.824</b>	<b>0.812</b>	-	0.442	0.180	0.255
	$[\text{Na}^+]^\dagger$	0.110	-0.137	-0.191	0.085	-0.205	-0.213	-0.151	-	0.001	<b>&lt;0.001</b>
	$[\text{Ca}^{2+}]^*$	0.193	0.269	0.193	0.232	0.251	0.315	0.261	0.612	-	<b>&lt;0.001</b>
	$[\text{Mg}^{2+}]^*$	-0.040	-0.228	-0.266	-0.001	-0.367	-0.323	-0.222	<b>0.918</b>	<b>0.640</b>	-

\* Sqrt transformed data

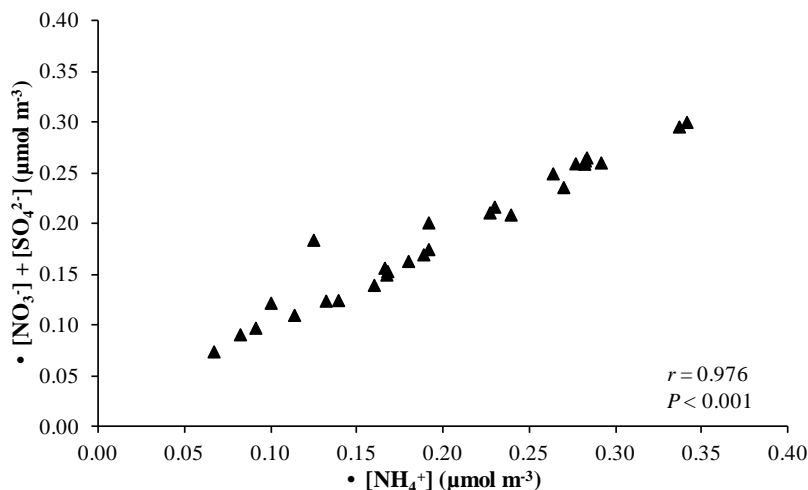
† Box-Cox transformed data

The second strongest correlation was between  $[\text{Na}^+]$  and  $[\text{Mg}^{2+}]$  (PPMCC;  $r = 0.967$ ).  $[\text{Na}^+]$  was subjectively omitted from the preliminary ordination analysis, using instead  $[\text{Mg}^{2+}]$  and  $[\text{Ca}^{2+}]$  to represent atmospheric base cation concentrations at each site.  $[\text{Mg}^{2+}]$  was selected over  $[\text{Na}^+]$  because base cation deposition data were quantified using  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (DEFRA, 2011).

The third very high correlation was between  $[\text{NO}_2]$  and  $[\text{SO}_2]$  (PPMCC;  $r = 0.919$ ). Both compounds typically originate from combustion as separate entities and hence, it is likely that their strong co-variance is synonymous with



a shared origin rather than a specifically direct relationship between the two compounds.



**Figure 3.15** Relationship between  $[\text{NH}_4^+]$  and  $([\text{NO}_3^-] + [\text{SO}_4^{2-}])_{\text{NSS}}$  in atmospheric aerosol amongst the 28 survey sites. Pearson's Product Moment Correlation Coefficient is indicated. All values were square root transformed prior to analysis to obtain normality.

The number of climate variables was reduced earlier to annual mean minimum temperature and OI (Section 3.2.1), and hence, no further action was required for climatic factors. Thus, the shortened list of atmospheric chemistry variables produced from this work, together with those of the climate factors, was entered into a preliminary NMDS analysis for the trunks and branches (Section 4.6).

### 3.4 Discussion

Analysis of the climatic variables showed that all correlated well with the OI except mean annual and monthly temperatures. The poorest relationship was between OI and mean monthly minimum temperature. This observation was of

interest as the latter variable was used in the calculation of the OI and some level of autocorrelation might have been expected. Given the high correlation between the two temperature values (Figure 3.1) and the fact that they both measured minimum temperature, it was decided to include only one of them together with OI scores in subsequent ordination analyses. Mean monthly minimum temperature was chosen for inclusion subjectively, because it had the weakest correlation to the OI (Table 3.2).

Deposition data were excluded from the ordination analysis based on the assumption that the modelled values held would auto-correlate with the OI, as the climatic index was derived from a calculation that included number of rain days > 1 mm. Consequently, addition of the deposition data might result in the over-fitting of the model and cluttering of the ordination plots. A dummy NMDS ordination including the deposition values did show that this was the case.

By excluding the deposition data, only concentration data of the environmental chemical variables were included in the ordination analyses. Due to the absence of complete measured datasets for the environmental chemistry variables, modelled data were required to fill in missing values. This required an attempt to assess the validity of the modelled data against the measured data held. In most instances the modelled data was seen to correlate well with measured values, however, discrepancies were identified with [HNO<sub>3</sub>] and [base cations] that could have been due to either the model or the measuring technique (Section 3.2.1). The filling in of missing data with modelled values

resulted in the formation of a combination of measured, modelled and mixed measured and modelled atmospheric chemistry datasets for use in analysis.

Once the datasets were established for the concentration variables, these were reduced further by removing some very highly correlated (PPMCC;  $r > 0.90$ ) variables. The highest correlated variables were  $[\text{NH}_4^+]$ ,  $[\text{NO}_3^-]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ , and this finding is supported by work undertaken by van Breemen *et al.*, (1982) and van Herk *et al.* (2003<sup>a,b</sup>). The reason for the strong correlation of these three ionic compounds is likely to be due to the formation of  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4^{2-}$  in precipitation van den Heuvel & Mason (1963); ap Simon *et al.* (1987). The very strong correlation (PPMCC;  $r = 0.976$ ) shown between the molar sum of the anionic ( $[\text{NO}_3^-] + [\text{SO}_4^{2-}]_{\text{NSS}}$ ) and cationic ( $[\text{NH}_4^+]$ ) concentration values obtained in this study (Figure 3.14) lends credence to this. The strong correlations between these aerosols meant that only one of the three variables ( $[\text{NH}_4^+]$ ) could justifiably be used in the ordination analysis.

Based on the same principle of PPMCC analysis,  $[\text{Na}^+]$  was excluded due to its strong relationship with  $[\text{Mg}^{2+}]$  (PPMCC;  $r = 0.918$ ). However  $[\text{NO}_2]$  and  $[\text{SO}_2]$  were both included, despite having a very strong relationship (PPMCC;  $r = 0.919$ ). Their inclusion was based on two suppositions. Firstly, their wet deposited counterparts ( $[\text{NO}_3^-]$  and  $[\text{SO}_4^{2-}]_{\text{NSS}}$ ) had already been excluded; and secondly, the two compounds are reported to have different effects on lichen communities, as displayed in the cases of *Hypogymnia physodes* and *Lecanora conizaeoides* (Gadsdon *et al.*, 2010; Bates *et al.*, 2001). This led to the

formation of a reduced set of variables that were to be entered into the subsequent ordination analyses (Table 3.6).

**Table 3.6** List of variables selected to be entered into the ordination analysis of trunks and branches at surveyed sites.

Trunks			Branches		
Variable	Form of Data	NMDS Matrix	Variable	Form of Data	NMDS Matrix
Epiphytic species	Measured	Main	Epiphytic species	Measured	Main
OI	Modelled	Second	OI	Modelled	Second
Mean monthly min. temp.	Modelled	Second	Mean monthly min. temp.	Modelled	Second
[NH <sub>3</sub> ]	Measured	Second	[NH <sub>3</sub> ]	Measured	Second
[NH <sub>4</sub> <sup>+</sup> ]	Mixed	Second	[NH <sub>4</sub> <sup>+</sup> ]	Mixed	Second
[HNO <sub>3</sub> ]	Modelled	Second	[HNO <sub>3</sub> ]	Modelled	Second
[SO <sub>2</sub> ]	Mixed	Second	[SO <sub>2</sub> ]	Mixed	Second
[NO <sub>2</sub> ]	Modelled	Second	[NO <sub>2</sub> ]	Modelled	Second
[Ca <sup>2+</sup> ]	Modelled	Second	[Ca <sup>2+</sup> ]	Modelled	Second
[Mg <sup>2+</sup> ]	Modelled	Second	[Mg <sup>2+</sup> ]	Modelled	Second
Bark pH	Measured	Second	Twig pH	Measured	Second
Tree girth	Measured	Second			
Bark rugosity	Measured	Second			

## **Chapter 4. Regional-Scale Survey Results**

### **4.1 Introduction**

This Chapter presents the results of the floristic survey and associated biological measurements. It also includes an assessment of the success of surveying trees within the target range of 1 km from each monitoring station, and an evaluation of the effectiveness of sample size in covering the significant proportion of species richness (SR) at survey sites. Reviewing surveyed tree distance from monitoring stations was required as consideration needed to be given to the high spatial variability of  $[\text{NH}_3]$ . The proportion of total species recorded at each site on surveyed trees was critical to enable both understanding of the survey work limitations and to provide some guidance on the minimum number of trees needing to be surveyed for a reliable estimation of scores in the bio-monitoring scheme.

The results of the ordination analyses for both the trunk and branch surveys were also reviewed. This included exploratory analysis on the epiphytic community composition, and the environmental variables that were superimposed on top of the plotted species dissimilarity matrix. From this a preliminary evaluation of potential indicator species was undertaken prior to the indicator species analysis (Chapter 5).

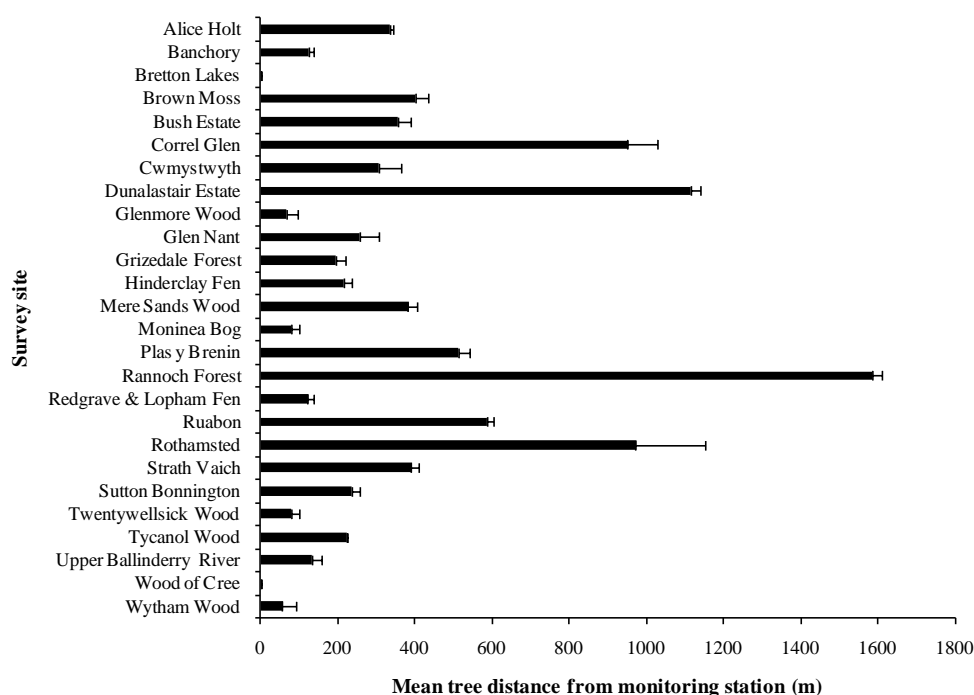
### **4.2 Phorophyte Assessment**

A total of 155 tree boles (49 *Betula*; 106 *Quercus*) and 300 branches (98 *Betula*; 202 *Quercus*) were surveyed across the 28 sites. Branches were not

surveyed at Twentywellsick Wood due to their inaccessibility. The five regional-scale survey sites where both *Betula* and *Quercus* were surveyed (Brown Moss, Correl Glen, Glen Nant, Hinderclay Fen and Redgrave and Lopham Fen) formed a northwest-southeast gradient across the UK (Figure 2.3). Although the specific locations did not segregate the oceanicity and [NH<sub>3</sub>] variables, it did provide a more robust means to evaluate the effect of phorophyte species on community composition, and subsequently, the scoring system to be used in the bio-monitoring scheme (Sections 4.6 and 5.2).

Mean tree distance from the [NH<sub>3</sub>] monitoring stations in all the regional-scale survey sites was within the designated 1 km radius of a monitoring station, with the exception of two locations. The Dunalastair Estate and Rannoch Forest survey locations were 1114 and 1586 m respectively from their respective air quality monitoring stations (Figure 4.1). Both sites were associated with the lowest mean monthly [NH<sub>3</sub>] values in the survey (Dunalastair Estate =  $0.04 \mu\text{g m}^{-3} \pm 0.01$ ; Rannoch Forest =  $0.03 \mu\text{g m}^{-3} \pm 0.01$ ), and did not have any sources of N emission within the vicinity. Consequently, the extended distance of these survey sites from the appropriate monitoring stations was not deemed to be a complication. In addition to this, the positions of certain individual trees at Correl Glen and Rothamsted were also observed to be beyond the target 1 km zone (Figure 4.1), with three trees at Correl Glen being located ca. 1204 m, and two Rothamsted trees being ca. 1386 m from the monitoring stations. As in the case of the previous two sites, no NH<sub>3</sub> point sources were identified either between the monitored trees, or between the entire survey area and monitoring station. Two sites (North Wyke and Yarner

Wood) were omitted from the calculation of mean tree distance (and also Figure 4.1) due to lack of GPS data, but *in-situ* estimates placed both survey sites within 300 m of their respective monitoring stations.



**Figure 4.1** Mean tree distance from monitoring station for 26 of the 28 regional-scale sites.

### 4.3 Tree Trunk Rugosity

Only 31 of the 49 surveyed *Betula* tree boles were reviewed due to the inability to obtain *in-situ* furrow measurements, mainly due to the presence of smooth, papery bark on a number of the aspects of some trees. A single *Betula* tree at two sites (Redgrave and Lopham Fen and Ruabon), and all of the trees at four locations (Brown Moss, Correl Glen, Hinderclay Fen and Moninea Bog) were affected in this way. A total of 105 of the 106 *Quercus* trunks surveyed were reviewed, the one exception being a tree from Glenmore Wood.

Tree trunk rugosity, as defined by the mean of the four Bt measurements taken at each aspect of the bole (Section 2.8.2), was plotted against the gbh measurement for each tree. The hypothesised negative trend in bark topography (Bt) score (Section 2.8.2) relative to tree age, as estimated by girth at breast height (gbh), was observed. This relationship was weak but significant for *Quercus*, although not significant for *Betula* (Figure 4.2). Because of this poor correlation between Bt and gbh both variables were entered into the ordination analysis for the trunk data. A comparison between the Bt values for *Betula* and *Quercus* with a gbh between 0.75 and 2.00 m showed no significant difference between the two tree genera (Table 4.1).

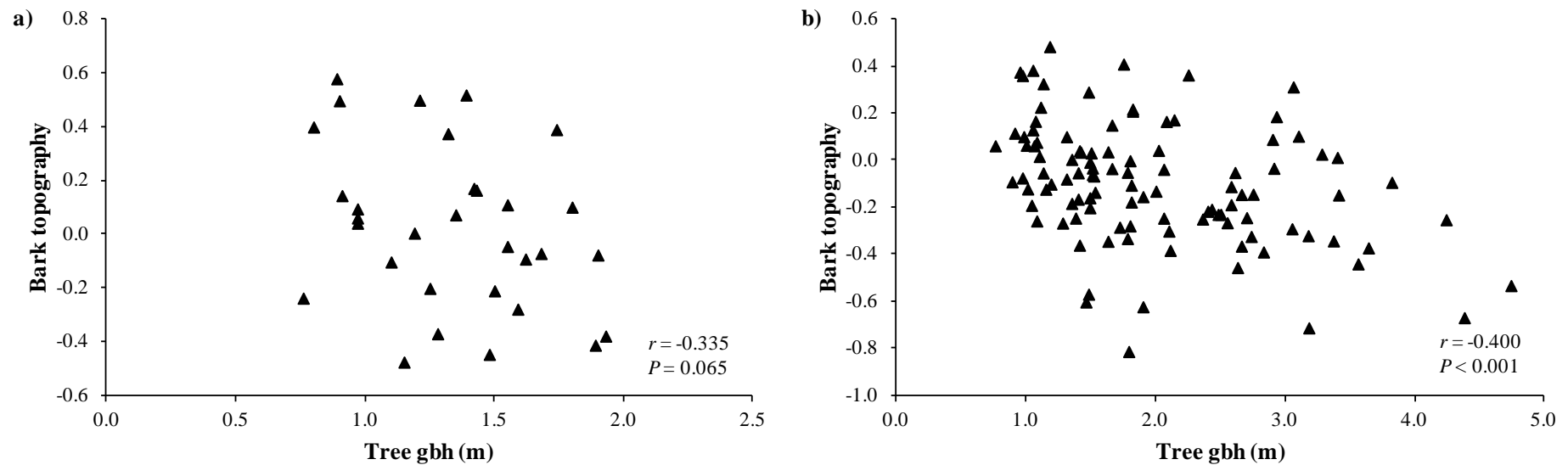
**Table 4.1** Comparison of bark topography on the boles of *Betula* and *Quercus* with a gbh of 0.75-2.00 m at the 28 survey sites. No significant difference (One-Way ANOVA;  $P = 0.209$ ) in topography was evident between the two tree genera.

Source	DF	SS	MS	F	$P$
Bark Topography	1	0.1166	0.1166	1.60	0.209
Error	92	6.7011	0.0728		
Total	93	6.8176			
S = 0.2699 R-Sq = 1.71% R-Sq(adj) = 0.64%					

Bark Topography	$n$	Mean	SD
<i>Betula</i> Bt	31	0.0256	0.3043
<i>Quercus</i> Bt	63	-0.0493	0.2516
Pooled SD			0.2699





**Figure 4.2** Relationship between tree girth at breast height (gbh), as defined by a set distance of 1.5 m from ground level, and bark topography on (a) *Betula* and (b) *Quercus* trees. Correlation co-efficient values calculated, using Box-Cox transformed tree girth data in the case for *Quercus*, showed a weak negative correlation. No significant correlation was found between topography and tree girth for *Betula* trees, although a negative trend was observed.

#### 4.4 *Quercus* Branch Length Analysis

Mean lengths of the two age classes of branches, 1-5 and 6-10 years, were compared for *Quercus* surveyed from 20 sites. Branches from two sites where *Quercus* was surveyed (Twentywellsick Wood and Yarner Wood) were excluded from the analysis due to lack of the appropriate data. The mean length of the 6-10 year age class was 56.34 cm ( $\pm 1.85$ ), 16.20 cm longer than that of the 1-5 year age class, which was 40.15 cm ( $\pm 1.48$ ); these differences in length were highly significant (Table 4.2).

**Table 4.2** The results of a one-way ANOVA to compare lengths of *Quercus* branches at 20 UK sampling sites following  $\log_{10}$  transformation.

Source	DF	SS	MS	F	P
Branch Age	1	2.3457	2.3457	59.38	0.001
Error	382	15.09	0.0395		
Total	383	17.4361			
S = 0.1988 R-Sq = 13.45% R-Sq(adj) = 13.23%					

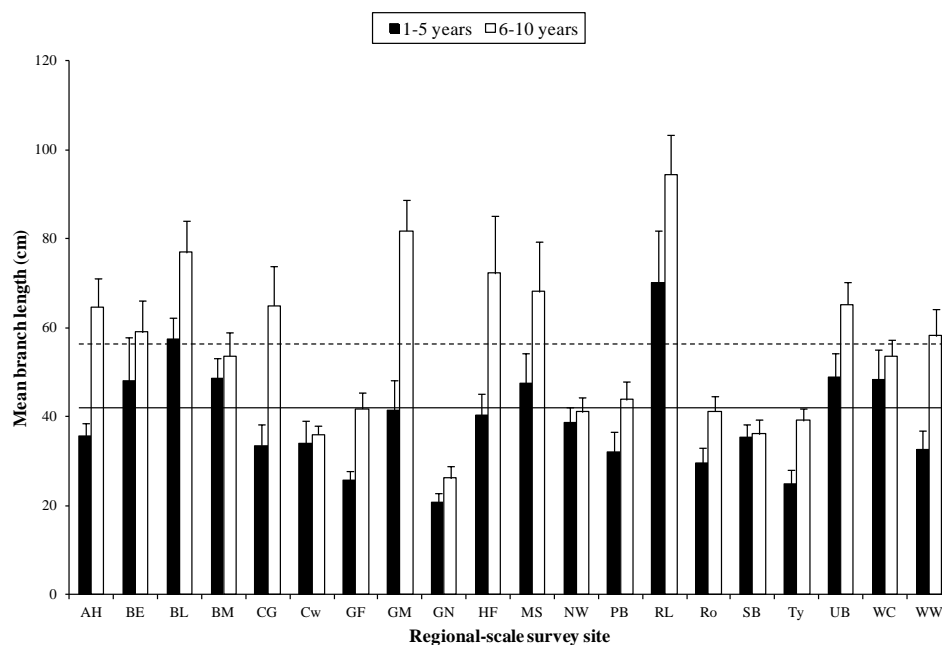
Branch Age Class	n	Mean	SD
1-5 years	192	1.5537	0.2086
6-10 years	192	1.7100	0.1883
Pooled SD			0.1988

Inter-site variation in branch length was significant for both branch sections (Table 4.3), with branch length variation being slightly higher for the 1-5 year age class than in the 6-10 year category (co-efficient of variation = 50.93% and 45.43% respectively). Mean branch length of the 1-5 year age class ranged from 20.75 cm ( $\pm 3.08$ ) at Glen Nant to 70.05 cm ( $\pm 11.84$ ) at Redgrave and Lopham Fen. The range of branch lengths for the 6-10 age class varied between 26.21 cm ( $\pm 2.64$ ) at Glen Nant to 94.45 cm ( $\pm 9.05$ ) at Redgrave & Lopham Fen respectively (Figure 4.3). The variability in length values

provided evidence of differences in tree growth rates between sites, and highlights a difficulty in deriving a definitive length measure of branch age.

**Table 4.3** Results of a one-way ANOVA to compare mean *Quercus* branch lengths across 20 UK sampling sites following  $\log_{10}$  transformation for (a) 1-5 year old branches and (b) 6-10 year old branches.

a)	Source	DF	SS	MS	F	P
	Site	19	2.709	0.1426	4.37	0.001
	Error	172	5.61	0.0326		
	Total	191	8.3151			
	S = 0.1805 R-Sq = 32.58% R-Sq(adj) = 25.13%					
b)	Source	DF	SS	MS	F	P
	Site	19	3.249	0.171	8.34	0.001
	Error	172	3.53	0.0205		
	Total	191	6.7753			
	S = 0.1432 R-Sq = 47.95% R-Sq(adj) = 42.20%					



**Figure 4.3** Branch length of *Quercus* at sampling sites for two branch age classes: 1-5 years (black columns) and 6-10 years (grey columns). Plotted values represent the mean lengths across all sites for 1-5 years (solid line) and 6-10 years (broken line).

#### 4.5 Epiphytic Sampling

Epiphytic sampling was undertaken at all 28 sampling sites. Five tree boles were sampled at 23 sites at which either *Betula* or *Quercus* were surveyed, and 10 branches at 22 sites (no branches were surveyed at Twentywellsick Wood). Eight trees were surveyed at the remaining five sites where both tree genera were surveyed due to time constraints. At these locations three *Betula* and five *Quercus* boles (six and 10 branches respectively) were surveyed at three sites (Brown Moss, Hinderclay Fen and Redgrave and Lopham Fen); and five *Betula* and three *Quercus* (10 and six branches respectively) at the remaining two (Correl Glen and Glen Nant).

To address concerns about the sufficiency of the sample number, the effect of increasing the number sampling units (trunks or branches) on cumulative SR was examined for both tree species at each site. This was done by plotting the cumulative SR against number of sampling units at each site and fitting a best fit line to each in Sigmaplot 11.0. Lines were fitted using a sigmoidal curve in most cases; however, in a few instances a power or linear line was fitted for optimum results. Asymptotic curves were observed in only 11 of the 33 of the trunk surveys (Appendix D), and in three of these instances it occurred where only three trees were surveyed. This suggested that at a large number of survey sites many more species would have been recorded if more trunks had been surveyed. Conversely, asymptotic curves were noted in 26 of the 32 sites for the branches, including two of the five sites where only six branches were surveyed (Appendix E).

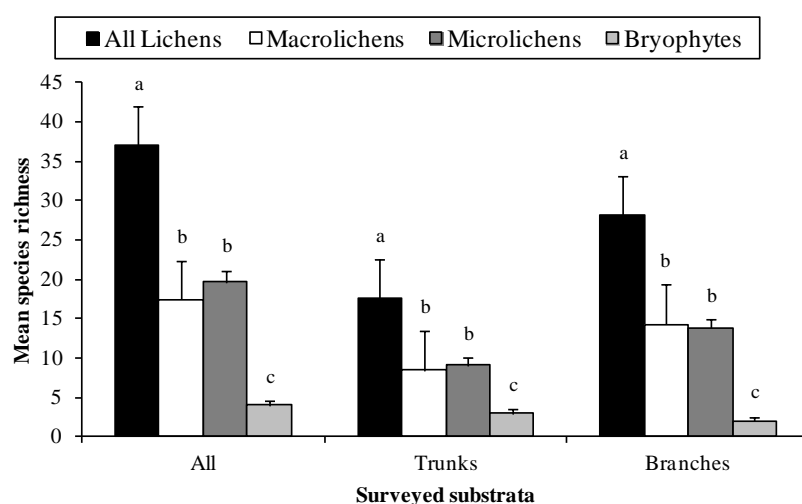
#### 4.6 Epiphytic Species Richness

A total of 169 epiphytes were identified to specific level across the 28 sites (Appendix F); 153 lichens (64 macrolichens and 89 microlichens) and 16 bryophytes (10 Bryophyta and 6 Marchantiophyta). A number of indeterminate lichen species remained following attempts at identification. All except two of these were identified to generic level. Several specimens of *Xanthoria ucrainica* S.Y. Kondr. (1997) were identified in the branch survey during laboratory identification. Due to concerns that other individuals were potentially overlooked and misidentified as *X. candelaria* (L.) Th. Fr. (1861) the two species were aggregated into the taxonomic group *X. candelaria* s.l., which included both these species and *Xanthoria ulophyllodes* Räsänen (1931).

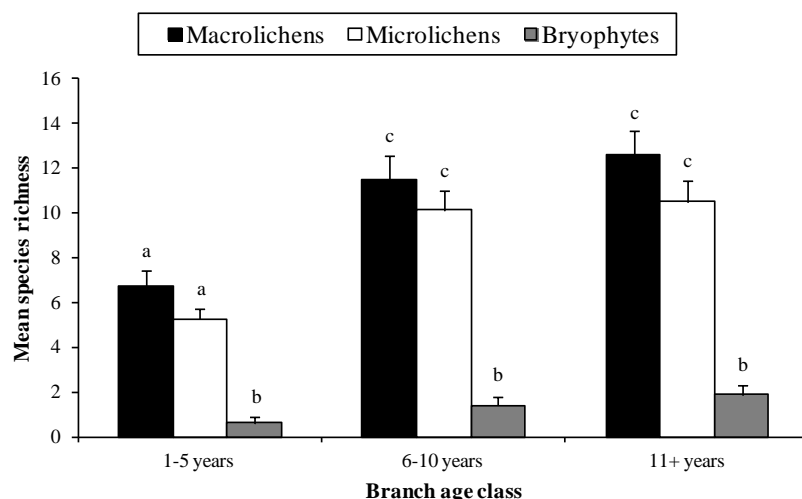
Species richness of lichens was greater than that of bryophytes (one-way ANOVA;  $P < 0.001$ ) for both trunks (SR = 18; bryophyte SR = 3) and branches (lichen SR = 28; bryophyte SR = 2) (Figure 4.4). The separation of lichens into macrolichens and microlichens did not alter this finding as mean numbers of both were observed to be significantly higher than bryophytes. Total lichen SR was significantly (One-Way ANOVA;  $P < 0.05$ ) higher on the branches than the trunks, and this was reflected in both the macro- and microlichen groupings. No significant difference in SR between microlichens and macrolichens was observed on either the trunks or the branches, despite the overall mean SR of microlichens being greater. One further observation made on lichen SR was that, although mean microlichen SR was greater both overall and on trunks, macrolichen SR was slightly higher on branches (Figure 4.4).

No significant difference (One-Way ANOVA;  $P > 0.05$ ) in SR was observed for bryophytes on trunks and branches.

Mean SR of both lichens and bryophytes increased with branch age (Figure 4.5). However, not all differences between branch age classes were significant. Species richness of both lichens and bryophytes were similar on the two older branch age classes (6-10 and 11+ years), but mean SR values for both macro- and microlichens were significantly lower on the 1-5 year old branches (Figure 4.5).



**Figure 4.4** Comparison of species richness of the three epiphyte groups (macrolichens, microlichens and bryophytes) surveyed on the tree boles and branches of *Betula* and *Quercus* at the 28 UK study sites. Letters indicate significant differences ( $P < 0.05$ ) using One-Way ANOVA on Box-Cox transformed data with a Tukey pairwise *post-hoc* test. Columns with the same letter are not significantly different at the  $P < 0.05$  level (one-way ANOVA;  $n = 28$  and  $27$  for trunks and branches respectively). Error bars represent the standard error of the mean.



**Figure 4.5** Comparison of mean species richness of the three epiphytic groups macrolichens, microlichens and bryophytes recorded along three age classes of branches at 28 UK survey sites. Letters indicate significant differences ( $P < 0.05$ ) both within and between age classes using a One-Way ANOVA on Box-Cox transformed data with a Tukey pairwise *post-hoc* test. Columns with the same letter are not significantly different at the  $P < 0.05$  level (one-way ANOVA;  $n = 27$ ). Error bars represent the standard error of the mean.

Mean macrolichen SR on branches was greater than that of microlichens (Figure 4.5). However, mean macrolichen SR for both trunks and branches was lower than microlichen SR (Figure 4.4), although this difference was not significant. The total number of microlichens recorded was uniformly higher than that of macrolichens, both for trunks and branches. This suggests that there was greater variation in microlichen species composition than that of macrolichens between trunks and branches. The mean percentage of macrolichens that occur on both trunks and branches for all sites was 31.2%, compared to 17.1% for microlichens, a difference of 14.1%. However, at five locations (Bretton Lakes, Dunalastair Estate, Mere Sands Wood, Redgrave and Lopham Fen and Strath Vaich) this difference was  $> 30\%$ . At Wood of Cree

none of the 30 microlichen species found were recorded on both trunks and branches.

#### **4.7 Exploratory Analysis of the Regional-Scale Survey Data**

Relationships between lichen communities and environmental variables were investigated using Non-linear Multi-dimensional Scaling (NMDS). The number of epiphytes used in the main matrices for the ordination analyses was reduced to a combined list of 103 lichen and bryophyte species for trunks and branches (Appendix G). This was achieved by removing species recorded on either trunks or branches that were present at less than three survey sites. These less well distributed species were excluded to prevent causing noise in the ordination analysis (McCune & Grace, 2002). The second set of matrices used in the analyses were comprised of the climate and atmospheric variables identified in Table 3.6 together with substratum pH and phorophyte species (as qualitative data) for branches, with the same variables plus gbh and rugosity for trunks.

##### **4.7.1 Initial Analyses and *Post-hoc* Testing**

The initial ordinations that were run with a stress test for trunks and branches and step down in dimensionality from six dimensions recommended a one-dimensional solution (minimum stress = 19.690; final instability = 0) for trunks (Table 4.4a) and a three-dimensional solution (minimum stress = 16.050; final instability = 0) for branches (Table 4.4b). The one-dimensional solution for trunks was provided by the model as the program selects a solution with a minimum stress < 20 if the reduction in minimum



stress between dimensions is  $< 5$  (Peck, 2010). Two problems arose from this solution. Firstly, the maximum level of stress at which an ordination can be perceived as acceptable under Clarke's rule of thumb (Clarke, 1993) is 20. Secondly, to enable a visual plotting of the trunk ordination at least two dimensions were required. A three-dimensional solution was subjectively selected (minimum stress = 13.630; final instability = 0) to resolve this matter. The reasons for selecting a three-dimensional solution over a two-dimensional one were based fundamentally upon the accumulative effect of minimum stress reduction through the two dimensions and the greater reduction in minimum stress observed between the two and three-dimensional solutions than between the one and two-dimensional solutions (Table 4.4a). The additive effect of selecting a three-dimensional solution resulted in a better overall minimum stress of 13.630, increased the proportion of variance explained in the model, and produced a lower  $P$ -value. In addition to this, the three-dimensional solution still limited the axes of the ordination plot to an interpretable level. The three-dimensional solution recommended for the branch epiphyte ordination was accepted for this ordination.

The consistency of the ordination results was confirmed in the results of the Mantel Tests between the five three-dimensional NMDS ordination analyses for both the trunk and branch surveys, all of which produced significant relationships ( $P < 0.001$ ). Two-dimensional graphs of the ordinations representing all possible pairings of the three axis plots were reviewed in consultation with three-dimensional ones generated in PC-Ord<sup>TM</sup>. The two-dimensional representations displaying the clearest accurate image of the

overlaid environmental variables with the species dissimilarity plots were then selected for visualisation of the trunk and branch ordinations. These were axes 1 and 2 for the trunk ordination (Figures 4.6-4.7; Table 4.5) and axes 1 and 3 for the branches (Figures 4.8-4.9; Table 4.6).

**Table 4.4** NMDS stress test of dimensionality for (a) trunk epiphyte surveys trunks and (b) branches in PC-Ord<sup>TM</sup>. Three-dimensional solutions were selected in both instances, despite a conflict with the recommended dimensionality (bold type).

a)	Stress in Real Data			Stress in Randomised Data			
	250 runs			Monte Carlo Test 250 runs			
Axes	Min.	Mean	Max.	Min.	Mean	Max.	P
<b>1</b>	<b>19.690</b>	<b>19.932</b>	<b>48.540</b>	<b>0.000</b>	<b>41.470</b>	<b>48.053</b>	<b>0.0319</b>
2	17.550	18.296	19.660	0.000	29.745	31.967	0.0319
3	13.630	13.922	14.807	0.001	22.858	24.456	0.0199
4	10.748	10.996	11.646	0.004	18.408	19.706	0.0199
5	8.922	9.080	9.653	0.003	15.331	16.361	0.0199
6	7.571	7.728	7.987	0.007	13.085	13.916	0.0199

b)	Stress in Real Data			Stress in Randomised Data			
	250 runs			Monte Carlo Test 250 runs			
Axes	Min.	Mean	Max.	Min.	Mean	Max.	P
1	35.032	38.651	48.610	32.974	51.752	53.874	0.0159
2	21.679	23.207	25.516	31.983	35.019	36.364	0.0040
<b>3</b>	<b>16.050</b>	<b>16.326</b>	<b>17.499</b>	<b>26.054</b>	<b>26.771</b>	<b>27.609</b>	<b>0.0040</b>
4	12.852	13.054	13.745	21.224	23.104	27.190	0.0040
5	11.023	11.141	11.507	17.743	20.489	23.724	0.0040
6	9.687	9.762	10.021	15.268	17.036	21.243	0.0040

#### 4.7.2 Observations on Ordination Analyses

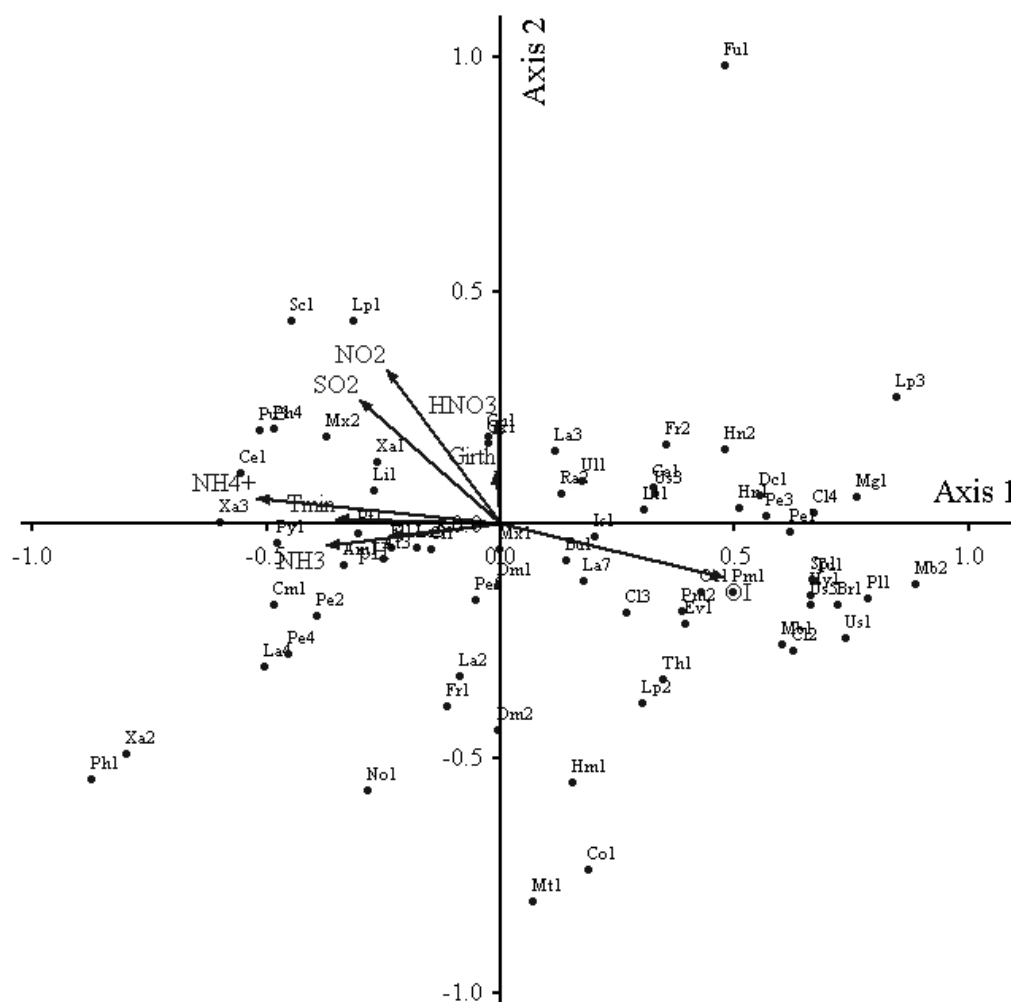
Two principle variable groupings were observed from both trunk and branch ordinations. One of these was [NO<sub>2</sub>] and [SO<sub>2</sub>] and the other [NH<sub>3</sub>], [NH<sub>4</sub><sup>+</sup>] and substrate pH. This outcome corroborates the results of earlier correlation analyses of the environmental chemistry variables (Section 3.2.5). The other

notable trend was the general gradation of the environmental chemistry variables in the opposing direction to the oceanicity index (OI), with  $[\text{NH}_4^+]$  appearing to be negatively correlated to the OI (Figures 4.6-4.9). The only exceptions to this trend were the base cation concentrations, most notably  $[\text{Mg}^{2+}]$  (Figures 4.8 and 4.9).

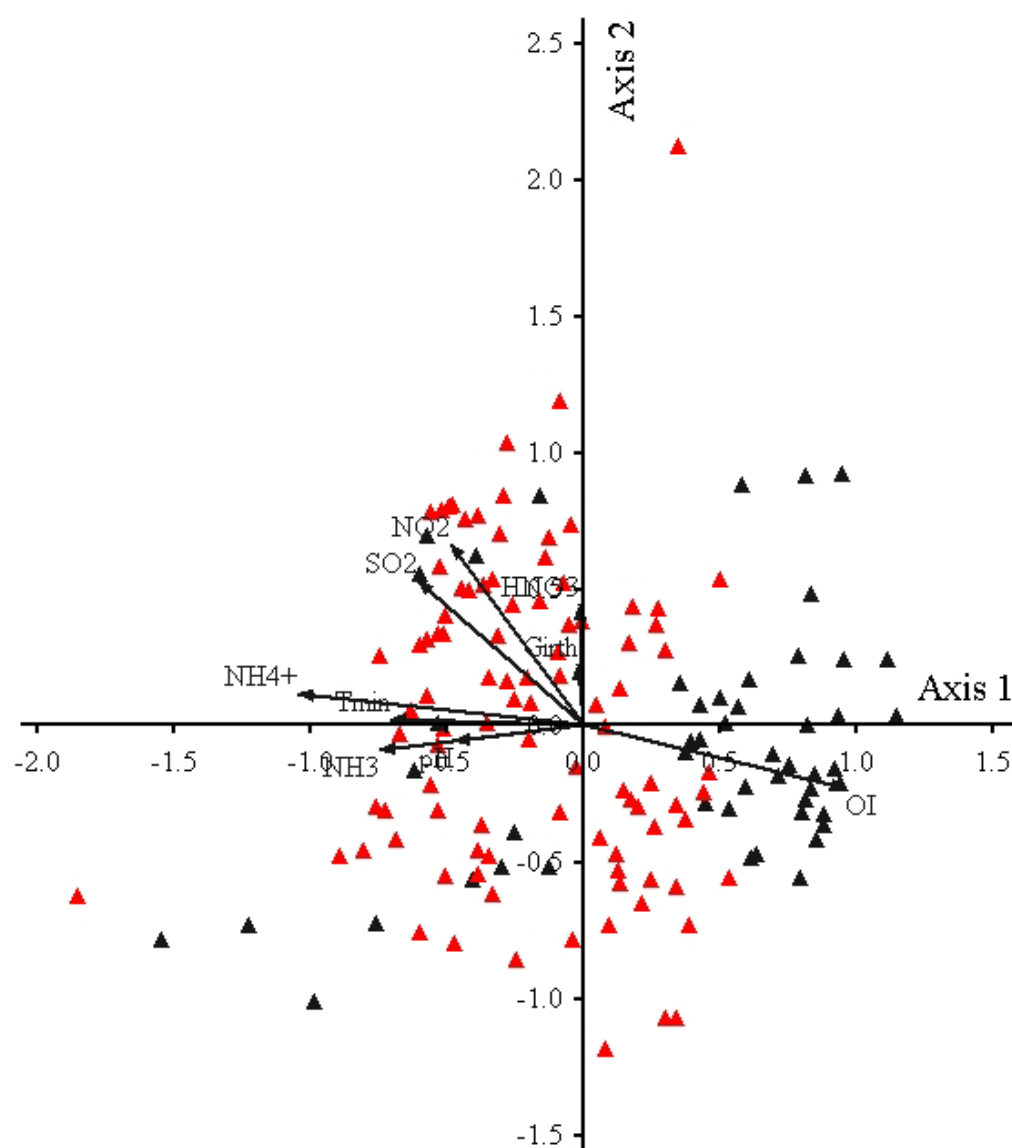
The species distributions in the ordination plots for trunks and branches displayed some consistent groupings. *Amandinea punctata*, *Physcia adscendens* and *Xanthoria parietina* were positioned near the end of the  $[\text{NH}_3]$ - $[\text{NH}_4^+]$  gradient for both trunks and branches, a consistent with a nitrophilic ecology; whilst *Bryoria fuscescens*, *Lepraria lobificans*, *L. rigidula*, *Mycoblastus fucatus*, *Parmelia saxatilis*, *Pertusaria albescens* var. *albescens*, *Platismatia glauca* and *Usnea* sp., occurred at the opposite end in close proximity to the OI gradient (Figures 4.6 and 4.8).

Other species found in the  $[\text{NH}_3]$ - $[\text{NH}_4^+]$  group of species included *Candelariella reflexa*, *Cliostomum griffithii*, *Pertusaria albescens* var. *corallina*, *P. hymenea*, *Lecanora confusa*, *Physcia tenella*, *Punctelia subrudecta*, *Pyrrhospora quernei* and *Xanthoria polycarpa* on the trunks (Figure 4.6); and *Arthonia radiata*, *Lecanora carpinea*, *L. persimilis*, *Lecidella elaeochroma*, *Physcia stellaris*, *Ramalina canariensis* and *R. fraxinea* on branches (Figure 4.8). Additional potential nitrophobic indicators included *Cladonia squamosa*, *C. polydactyla*, *Evernia prunastri*, *Megalania pulverea*, *Mycoblastus sanguinarius*, *Ochrolechia androgyna*, *Parmelia sulcata* and *Pertusaria amara* on the trunks (Figure 4.6; Table 4.5); and *Buellia*

*griseovirens*, *Graphis elegans*, *Mycoporum antecellens*, *Parmotrema perlatum*, *P. reticulatum* and *Pseudevernia furfuracea* on branches (Figure 4.8).



**Figure 4.6** The two-dimensional ordination plot of a three-dimensional NMDS analysis for tree trunk epiphyte survey data based on the first and second axes (see Appendix G for descriptive of species abbreviations).



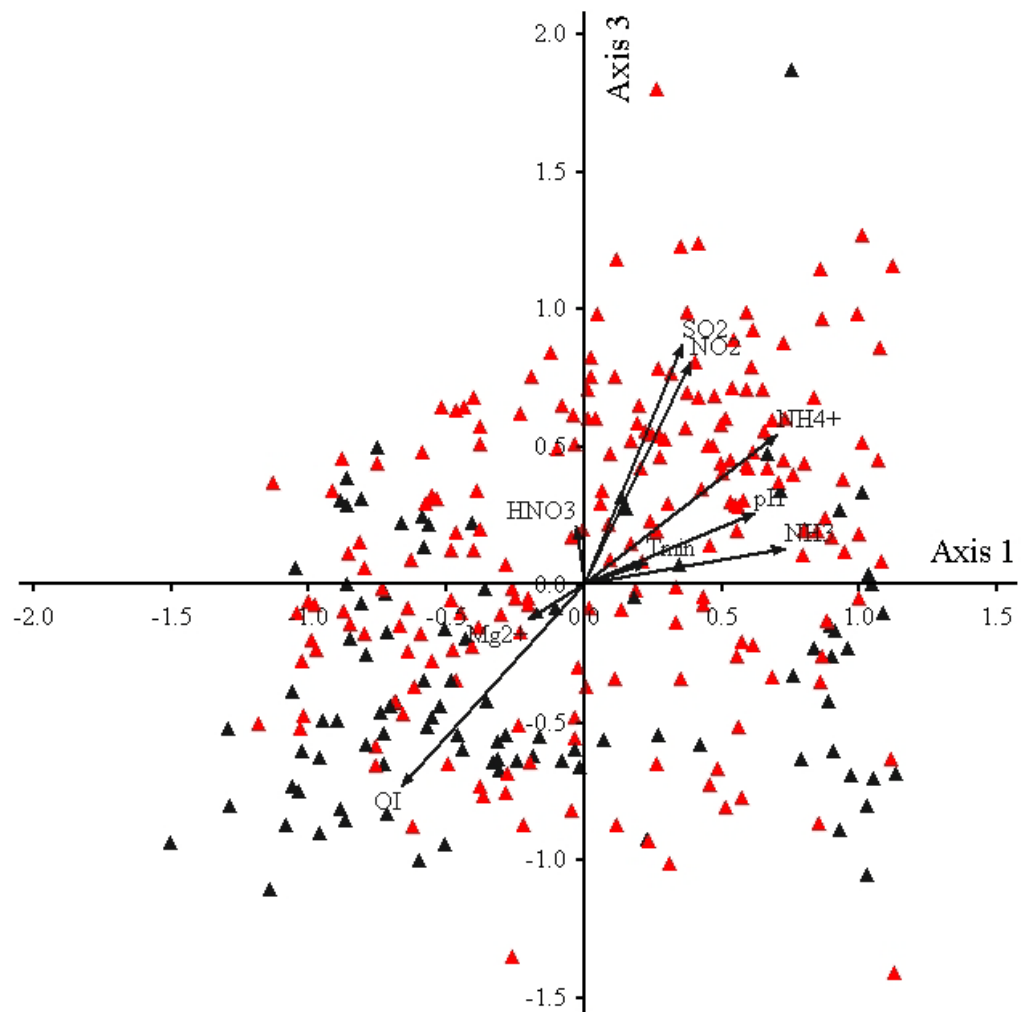
**Figure 4.7** The two-dimensional ordination plot of a three-dimensional NMDS analysis for tree trunk epiphyte survey data, based on the first and second axes, showing spatial separation of the two tree genera *Betula* spp. (black) and *Quercus* spp. (red).

**Table 4.5** Proportion of variance explained for the trunk data ordination plots (Figures 4.6 and 4.7).

a)	Axis	$r^2$ value	
		Increment	Cumulative
	1	0.218	0.218
	2	0.160	0.378
	3	0.132	0.510

b)	Axis Pair	r	Orthogonality
			(%)
	1 v 2	-0.003	100.0
	1 v 3	0.300	91.0
	2 v 3	-0.210	95.6





**Figure 4.9** The two-dimensional ordination plot of a three-dimensional NMDS analysis for tree branch epiphyte survey data, based on the first and third axes, showing spatial separation of the two tree genera *Betula* spp. (black) and *Quercus* spp. (red).

**Table 4.6** Proportion of variance explained for the branch data ordination plots (Figures 4.8 and 4.9).

a)	Axis	$r^2$ value	
		Increment	Cumulative
	1	0.348	0.348
	2	0.177	0.525
	3	0.207	0.732

b)	Axis Pair	r	Orthogonality
			(%)
	1 v 2	-0.008	100.0
	1 v 3	0.303	90.8
	2 v 3	0.026	99.9

## 4.8 Identification of Indicator Species for [NH<sub>3</sub>]

Following the exploration of the survey data, and preliminary assessment of potential indicator species in NMDS, Indicator Species Analysis (ISA) was undertaken in PC-Ord<sup>TM</sup>. This section describes the ISA results and identifies a short-list of key indicator species that was produced based on the methodology and criteria defined in Section 2.13.2. These species were then used in Chapter 5 to calculate bio-monitoring scores for the four different Frequency-based Lichen Acidophyte Nitrophyte (FL<sub>AN</sub>) methods described in Section 2.13.3.

### 4.8.1 Indicator Species Analysis Results

The ISA identified 32 trunk and 48 branch indicator species. Trunk indicators were comprised of 29 lichens (11 micro- and 18 macrolichens) and three bryophytes (one Bryophyta and two Marchantiophyta). In total, 23 of the indicators were defined as nitrophobic (21 lichens and 2 bryophytes), and nine as nitrophilic (eight lichens and one Marchantiophyta). Of all the lichen indicators identified, 18 were previously recorded as such (Table 4.7), and the current findings for 16 of these species concurred with established understanding. The two exceptions were *Chaenotheca ferruginea* and *Lepraria incana*. Both of these lichens were reported to be acidophytic by van Herk (1999) but were found to be significantly nitrophilic in the trunk analysis (Table 4.8).

The reason for the disparity is not known in the case of *C. ferruginea*, and indicator species analysis was only undertaken on the trunks for this species. The ordination analysis of the trunk data shows that the presence of this species is associated with tree girth, rather than [NH<sub>3</sub>]. One possible explanation for



the discrepancy is that the low sample number (i.e. *C. ferruginea* was only recorded on the trunks at four sites) and preference for more rugose (and potentially older) trees may have influenced the analysis.

**Table 4.7** Lichen species listed as being either acidophytic (nitrophobic) or nitrophytic (nitrophilic) indicators from previous studies.

Acidophyte*	Source	Nitrophyte*	Source
<i>Bryoria</i> spp.	van Herk <i>et al.</i> (2003 <sup>ab</sup> )	<i>Caloplaca citrina</i>	van Herk (1999)
<i>Chaenotheca ferruginea</i>	van Herk (1999)	<i>Caloplaca holocarpa</i>	van Herk (1999)
<i>Cladonia</i> spp.	van Herk (1999)	<i>Candelariella aurella</i>	van Herk (1999)
<i>Evernia prunastri</i>	van Herk (1999)	<i>Candelariella reflexa</i>	van Herk (1999)
<i>Flavoparmelia caperata</i>	Wolseley (2005 <sup>a</sup> )	<i>Candelariella vitellina</i>	van Herk (1999)
<i>Hypocenomyce scalaris</i>	van Herk (1999)	<i>Candelariella xanthostigma</i>	van Herk (1999)
<i>Hypogymnia physodes</i>	van Herk (1999)	<i>Diploica canescens</i>	Wolseley (2005 <sup>a</sup> )
<i>Hypogymnia tubulosa</i>	van Herk (1999)	<i>Hyperphyscia adglutinata</i>	Wolseley (2005 <sup>a</sup> )
<i>Hypotrachyna revoluta</i>	van Herk (2002)	<i>Lecanora dispersa</i> s.l.	van Herk (1999)
<i>Imshaugia aleurites</i>	van Herk <i>et al.</i> (2003 <sup>ab</sup> )	<i>Lecanora muralis</i> †	van Herk (1999)
<i>Lecanora aitema</i>	van Herk (1999)	<i>Parmelina</i> spp.	Wolseley (2005 <sup>a</sup> )
<i>Lecanora conizaeoides</i>	van Herk (1999)	<i>Phaeophyscia nigricans</i> †	van Herk (1999)
<i>Lecanora pulicaris</i>	van Herk (1999)	<i>Phaeophyscia orbicularis</i>	van Herk (1999)
<i>Lepraria incana</i>	van Herk (1999)	<i>Physcia adscendens</i>	van Herk (1999)
<i>Ochrolechia microstictoides</i>	van Herk (1999)	<i>Physcia caesia</i> †	van Herk (1999)
<i>Parmelia saxatilis</i>	van Herk (1999)	<i>Physcia dubia</i>	van Herk (1999)
<i>Parmeliopsis ambigua</i>	van Herk (1999)	<i>Physcia tenella</i>	van Herk (1999)
<i>Placynthiella icmalea</i>	van Herk (1999)	<i>Physconia</i> spp.	Wolseley (2005 <sup>a</sup> )
<i>Platismatia glauca</i>	van Herk (1999)	<i>Rinodina gennarii</i>	van Herk (1999)
<i>Pseudevernia furfuracea</i>	van Herk (1999)	<i>Xanthoria calcicola</i> †	van Herk (1999)
<i>Trapeliopsis flexuosa</i>	van Herk (1999)	<i>Xanthoria candelaria</i>	van Herk (1999)
<i>Trapeliopsis granulosa</i>	van Herk (1999)	<i>Xanthoria parietina</i>	van Herk (1999)
<i>Tuckermanopsis chlorophylla</i>	van Herk (1999)	<i>Xanthoria polycarpa</i>	van Herk (1999)
<i>Usnea</i> spp.	van Herk (1999)		
<i>Vulpicida pinastri</i>	van Herk <i>et al.</i> (2003 <sup>ab</sup> )		

\* See Chapter 1 for comments on terminology

† Saxicolous spp.

*Lepraria incana* was recorded at 19 sites and assessed on both the trunks and branches. Despite being identified as a significantly nitrophilic species on the trunks ( $P = 0.029$ ), *L. incana* was identified as a nitrophobe on the branches, although this relationship was not significant ( $P > 0.05$ ). As a coloniser of microclimatic conditions typically associated with older phorophyte substrata

(Laundon, 1992), *L. incana* would not have been as evenly distributed along branches compared to other species (e.g. *Hypogymnia tubulosa*), and restriction of the lichen to the oldest branch sections at sites where it was recorded is a likely explanation for the lack of significance as a nitrophobe in the branch results. The NMDS analysis (Figure 4.6; Table 4.5) also showed that *L. incana* was associated more with [NO<sub>2</sub>] and, although this implies [NO<sub>2</sub>] tolerance of the species, it supports the notion that it is not necessarily [NH<sub>3</sub>] tolerant. The discrepancy between the result for trunks and established thinking could also be explained in part by the favourable microclimatic conditions of the substratum (Laundon, 1992). As a species that spreads by vegetative propagation, this might aid its persistence, making it an example of a relict species of past conditions on the trunks. Established individuals on trunks are probably more buffered against increases in substratum pH caused by [NH<sub>3</sub>] compared to branches due to the more acidic nature of trunk substratum.

The 48 branch indicators were comprised of 43 lichens (22 micro- and 21 macrolichens) and five bryophytes (three Bryophyta and two Marchantiophyta). In total 32 were nitrophobic (27 lichens and 5 bryophytes) and 16 nitrophilic (Table 4.9). Of the species defined as indicators in the ISA, 18 of the lichens were previously identified as such (Table 4.7), with all of the indicator types matching perfectly with established reports (Table 4.9) in each case.

**Table 4.8** Monte Carlo Test of significance for trunk indicator species based on the percentage of perfect indication (*IV*), as identified by indicator species analysis in PC-Ord<sup>TM</sup>. Species in bold are those previously identified as indicators. Red type indicates lichens in which the current analysis contradicts established understanding of species ecology.

Lichen Species	Indicator	<i>IV</i>	Mean	SD	<i>P</i>	B%fo <sup>*</sup>	Q%fo <sup>†</sup>	Q:B <sup>‡</sup>
<i>Arthonia spadicea</i>	Nitrophobe	7.1	3.2	1.29	0.020	0.00	18.18	N/A
<i>Bryoria fuscescens</i>	Nitrophobe	<b>22.5</b>	<b>7.7</b>	<b>1.96</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>0.00</b>	N/A
<i>Candelariella reflexa</i>	Nitrophile	17.7	6.9	1.86	0.001	18.18	18.18	1.0
<i>Chaenotheca ferruginea</i>	Nitrophile	<b>16.5</b>	<b>7.4</b>	<b>1.94</b>	<b>&lt; 0.001</b>	<b>9.09</b>	<b>13.64</b>	<b>1.5</b>
<i>Cladonia coniocraea</i>	Nitrophobe	15.7	10.9	2.31	0.039	36.36	27.27	0.8
<i>Cladonia polydactyla</i>	Nitrophobe	35.9	13.0	2.53	< 0.001	36.36	18.18	0.5
<i>Cladonia pyxidata</i>	Nitrophobe	15.0	6.6	1.91	< 0.001	18.18	18.18	1.0
<i>Cladonia squamosa</i>	Nitrophobe	12.7	4.9	1.60	0.001	9.09	9.09	1.0
<i>Cliostomum griffithii</i>	Nitrophile	36.0	17.5	2.81	< 0.001	36.36	54.55	1.5
<i>Hypogymnia physodes</i>	Nitrophobe	43.8	14.5	2.52	< 0.001	54.55	22.73	0.4
<i>Hypotrachyna revoluta</i>	Nitrophobe	18.9	12.8	2.58	0.028	36.36	27.27	0.8
<i>Lepraria incana</i>	Nitrophile	<b>37.4</b>	<b>28.3</b>	<b>2.95</b>	<b>0.009</b>	<b>54.55</b>	<b>68.18</b>	<b>1.3</b>
<i>Lepraria lobificans</i>	Nitrophobe	37.4	20.5	2.83	< 0.001	45.45	45.45	1.0
<i>Lepraria rigidula</i>	Nitrophobe	16.3	6.6	1.91	< 0.001	36.36	4.55	0.1
<i>Mycoblastus fucatus</i>	Nitrophobe	15.5	5.8	1.74	< 0.001	27.27	4.55	0.2
<i>Mycoblastus sanguinarius</i>	Nitrophobe	15.5	5.8	1.77	< 0.001	27.27	0.00	N/A
<i>Ochrolechia androgyna</i>	Nitrophobe	35.2	11.2	2.37	< 0.001	27.27	22.73	0.8
<i>Parmelia saxatilis</i>	Nitrophobe	52.2	16.8	2.77	< 0.001	54.55	31.82	0.6
<i>Parmelia sulcata</i>	Nitrophobe	18.5	12.8	2.63	0.038	45.45	31.82	0.7
<i>Physcia adscendens</i>	Nitrophile	17.7	7.0	1.98	< 0.001	9.09	13.64	1.5
<i>Platismatia glauca</i>	Nitrophobe	35.2	11.2	2.37	< 0.001	54.55	13.64	0.3
<i>Pyrrhospora quernea</i>	Nitrophile	13.2	7.2	2.02	0.012	0.00	27.27	N/A
<i>Sphaeophorus globosus</i>	Nitrophobe	18.3	6.6	1.87	< 0.001	18.18	13.64	0.8
<i>Thelotrema lepadinum</i>	Nitrophobe	28.9	10.5	2.33	< 0.001	36.36	22.73	0.6
<i>Tuckermanopsis cholorphylla</i>	Nitrophobe	16.9	6.2	1.80	< 0.001	36.36	0.00	N/A
<i>Usnea cornuta</i>	Nitrophobe	8.5	3.7	1.41	0.009	18.18	0.00	N/A
<i>Usnea subfloridana</i>	Nitrophobe	24.4	8.9	2.13	< 0.001	45.45	4.55	0.1
<i>Xanthoria parietina</i>	Nitrophile	21.5	8.3	2.18	< 0.001	27.27	22.73	0.8
<i>Xanthoria polycarpa</i>	Nitrophile	16.5	6.6	1.94	0.002	18.18	27.27	1.5
bryophyte Species	Indicator	<i>IV</i>	Mean	SD	<i>P</i>	B%fo <sup>*</sup>	Q%fo <sup>†</sup>	Q:B <sup>‡</sup>
<i>Dicranum scoparium</i>	Nitrophobe	42.4	16.0	2.68	< 0.001	45.45	31.82	0.70
<i>Frullania tamarisci</i>	Nitrophobe	13.1	8.9	2.11	0.050	27.27	13.64	0.50
<i>Calypoglia fissa</i>	Nitrophile	9.9	5.8	1.75	0.032	18.18	13.64	0.75

\* Percentage of *Betula* trees each epiphyte recorded on

† Percentage of *Quercus* trees each epiphyte recorded on

‡ Relative percentage frequency of epiphytic occurrence on *Quercus* and *Betula* tree

**Table 4.9** Monte Carlo Test of significance for branch indicator species based on the percentage of perfect indication (*IV*), as identified by indicator species analysis in PC-Ord™. Species in bold are those identified as indicators in previous studies.

Lichen Species	Indicator	<i>IV</i>	Mean	SD	<i>P</i>	B% <i>fo</i> <sup>*</sup>	Q% <i>fo</i> <sup>†</sup>	Q:B <sup>‡</sup>
<i>Amandinea punctata</i>	Nitrophile	4.2	2.1	0.78	0.024	0.00	23.81	N/A
<i>Anisomeridium biforme</i>	Nitrophile	7.7	4.1	1.02	0.005	0.00	23.81	N/A
<i>Arthonia radiata</i>	Nitrophile	23.7	10.9	1.60	< 0.001	27.27	57.14	2.1
<i>Arthopyrena punctiformis</i>	Nitrophobe	5.6	2.3	0.79	0.024	0.00	19.05	N/A
<b><i>Bryoria fuscescens</i></b>	<b>Nitrophobe</b>	<b>7.0</b>	<b>2.7</b>	<b>0.85</b>	<b>0.001</b>	<b>18.18</b>	<b>0.00</b>	<b>N/A</b>
<i>Buellia griseovirens</i>	Nitrophobe	3.5	1.6	0.64	0.025	27.27	0.00	N/A
<b><i>Candelariella reflexa</i></b>	<b>Nitrophile</b>	<b>12.1</b>	<b>7.0</b>	<b>1.34</b>	<b>0.005</b>	<b>0.00</b>	<b>52.38</b>	<b>N/A</b>
<b><i>Evernia prunastri</i></b>	<b>Nitrophobe</b>	<b>27.7</b>	<b>13.0</b>	<b>1.70</b>	<b>&lt; 0.001</b>	<b>27.27</b>	<b>66.67</b>	<b>2.4</b>
<i>Fuscidea lightfootii</i>	Nitrophobe	33.6	23.8	1.98	< 0.001	54.55	80.95	1.5
<i>Graphis elegans</i>	Nitrophobe	30.9	11.9	1.62	< 0.001	27.27	52.38	1.9
<b><i>Hypogymnia physodes</i></b>	<b>Nitrophobe</b>	<b>65.3</b>	<b>22.9</b>	<b>1.94</b>	<b>&lt; 0.001</b>	<b>63.64</b>	<b>52.38</b>	<b>0.8</b>
<b><i>Hypogymnia tubulosa</i></b>	<b>Nitrophobe</b>	<b>36.0</b>	<b>12.7</b>	<b>1.68</b>	<b>&lt; 0.001</b>	<b>54.55</b>	<b>42.86</b>	<b>0.8</b>
<b><i>Hypotrachyna revoluta</i></b>	<b>Nitrophobe</b>	<b>36.1</b>	<b>16.2</b>	<b>1.82</b>	<b>0.001</b>	<b>27.27</b>	<b>61.90</b>	<b>2.3</b>
<i>Lecanora chlarotera</i>	Nitrophile	43.8	34.6	1.89	< 0.001	90.91	100.00	1.1
<i>Lecanora expallens</i>	Nitrophobe	11.6	6.4	1.26	0.002	18.18	28.57	1.6
<i>Lecanora persimilis</i>	Nitrophile	14.1	5.1	1.14	< 0.001	27.27	23.81	0.9
<b><i>Lecanora pulicaris</i></b>	<b>Nitrophobe</b>	<b>41.5</b>	<b>13.3</b>	<b>1.69</b>	<b>&lt; 0.001</b>	<b>100.00</b>	<b>9.52</b>	<b>0.1</b>
<i>Lecanora symmicta</i>	Nitrophobe	12.7	7.4	1.38	0.004	36.36	42.86	1.2
<i>Lecidella elaeochroma</i>	Nitrophile	27.0	20.4	1.93	0.006	63.64	85.71	1.3
<i>Lepraria lobificans</i>	Nitrophobe	12.4	5.8	1.19	< 0.001	27.27	19.05	0.7
<i>Lepraria rigidula</i>	Nitrophobe	9.9	3.5	0.94	< 0.001	27.27	4.76	0.2
<i>Mycoblastus fucatus</i>	Nitrophobe	15.5	5.1	1.15	< 0.001	45.45455	9.52	0.2
<i>Mycoporum antecellans</i>	Nitrophobe	8.8	4.3	1.06	0.003	18.18	28.57	1.6
<i>Opegraph atra</i>	Nitrophile	16.2	7.4	1.36	< 0.001	18.18	23.81	1.3
<b><i>Parmelia saxatilis</i></b>	<b>Nitrophobe</b>	<b>26.0</b>	<b>8.5</b>	<b>1.46</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>33.33</b>	<b>0.9</b>
<i>Parmelia sulcata</i>	Nitrophobe	63.8	29.0	2.00	< 0.001	72.73	76.19	1.0
<i>Pertusaria albescens</i> var. <i>albescens</i>	Nitrophobe	4.3	2.4	0.83	0.041	27.27	14.29	0.5
<i>Phaeographis smithii</i>	Nitrophile	9.6	3.7	1.00	< 0.001	9.09	9.52	1.0
<b><i>Physcia tenella</i></b>	<b>Nitrophile</b>	<b>48.1</b>	<b>26.1</b>	<b>1.96</b>	<b>&lt; 0.001</b>	<b>45.45</b>	<b>85.71</b>	<b>1.9</b>
<b><i>Platismatia glauca</i></b>	<b>Nitrophobe</b>	<b>59.5</b>	<b>17.2</b>	<b>1.81</b>	<b>&lt; 0.001</b>	<b>54.55</b>	<b>42.86</b>	<b>0.8</b>
<i>Porina aeana</i>	Nitrophobe	6.2	3.7	1.01	0.031	0.00	28.57	N/A
<b><i>Pseudevernia furfuracea</i></b>	<b>Nitrophobe</b>	<b>19.3</b>	<b>6.4</b>	<b>1.26</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>14.29</b>	<b>0.4</b>
<i>Punctelia subrudecta</i>	Nitrophile	20.4	10.8	1.61	< 0.001	9.09	47.62	5.2
<i>Ramalina fraxinea</i>	Nitrophile	6.6	3.3	0.92	0.005	0.00	23.81	N/A
<i>Scoliciosporum chlorococcum</i>	Nitrophile	33.9	20.9	1.91	< 0.001	54.55	66.67	1.2
<b><i>Usnea cornuta</i></b>	<b>Nitrophobe</b>	<b>8.9</b>	<b>4.9</b>	<b>1.12</b>	<b>0.004</b>	<b>27.27</b>	<b>19.05</b>	<b>0.7</b>
<b><i>Usnea flammea</i></b>	<b>Nitrophobe</b>	<b>15.5</b>	<b>5.1</b>	<b>1.15</b>	<b>&lt; 0.001</b>	<b>27.27</b>	<b>14.29</b>	<b>0.5</b>
<b><i>Usnea hirta</i></b>	<b>Nitrophobe</b>	<b>5.6</b>	<b>2.3</b>	<b>0.76</b>	<b>0.002</b>	<b>27.27</b>	<b>4.76</b>	<b>0.2</b>
<b><i>Usnea subfloridana</i></b>	<b>Nitrophobe</b>	<b>40.2</b>	<b>12.1</b>	<b>1.64</b>	<b>&lt; 0.001</b>	<b>45.45</b>	<b>28.57</b>	<b>0.6</b>
<b><i>Usnea wasmuthii</i></b>	<b>Nitrophobe</b>	<b>10.6</b>	<b>3.7</b>	<b>0.99</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>9.52</b>	<b>0.3</b>
<b><i>Xanthoria candelaria</i> s.l.</b>	<b>Nitrophile</b>	<b>12.7</b>	<b>7.0</b>	<b>1.33</b>	<b>0.002</b>	<b>45.45</b>	<b>38.10</b>	<b>0.8</b>
<b><i>Xanthoria parietina</i></b>	<b>Nitrophile</b>	<b>65.7</b>	<b>23.6</b>	<b>1.94</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>76.19</b>	<b>2.1</b>
<b><i>Xanthoria polycarpa</i></b>	<b>Nitrophile</b>	<b>43.1</b>	<b>15.8</b>	<b>1.79</b>	<b>&lt; 0.001</b>	<b>36.36</b>	<b>57.14</b>	<b>1.6</b>
bryophyte Species	Indicator	<i>IV</i>	Mean	SD	<i>P</i>	B% <i>fo</i> <sup>*</sup>	Q% <i>fo</i> <sup>†</sup>	Q:B <sup>‡</sup>
<b><i>Frullania tamarisci</i></b>	<b>Nitrophobe</b>	<b>15.2</b>	<b>4.7</b>	<b>1.10</b>	<b>&lt; 0.001</b>	<b>18.18</b>	<b>14.29</b>	<b>0.79</b>
<i>Frullania dilatata</i>	Nitrophobe	16.8	10.1	1.53	0.001	27.27	42.86	1.57
<i>Hypnum cupressiforme</i>	Nitrophobe	16.9	5.4	1.18	< 0.001	0.00	19.05	N/A
<i>Isoetecium myosuroides</i>	Nitrophobe	15.1	9.2	1.50	0.003	18.18	47.62	2.62
<i>Ulotia crispa</i>	Nitrophobe	30.2	13.7	1.73	< 0.001	27.27	47.62	1.75

\* Percentage of *Betula* trees each epiphyte recorded on

† Percentage of *Quercus* trees each epiphyte recorded on

‡ Relative percentage frequency of epiphytic occurrence on *Quercus* and *Betula* tree

#### **4.8.2 Comparison between ISA and NMDS**

Most of the indicator species identified in the ISA (Tables 4.8 and 4.9) were supported by the ordination analyses (Figures 4.6 and 4.8). Species that came out as potential candidates in the NMDS ordination analysis, but were not significantly confirmed as indicators of  $[\text{NH}_3]$  in the ISA included *Amandinea punctata* (ISA;  $IV = 1.6$ ;  $P = 0.8708$ ), *Physcia tenella* (ISA;  $IV = 5.8$ ;  $P = 0.1332$ ) and *Evernia prunastri* (ISA;  $IV = 15.1$ ;  $P = 0.0736$ ) on the trunks, and *Usnea filipendula* (ISA;  $IV = 2.0$ ;  $P = 0.2324$ ) and *Physcia adscendens* (ISA;  $IV = 6.1$ ;  $P = 0.1026$ ) on the branches. *E. prunastri* was observed to be a good indicator for both trunks and branches in the NMDS (Figures 4.6 and 4.8), despite no significant result being obtained in the indicator species analysis for trunks.

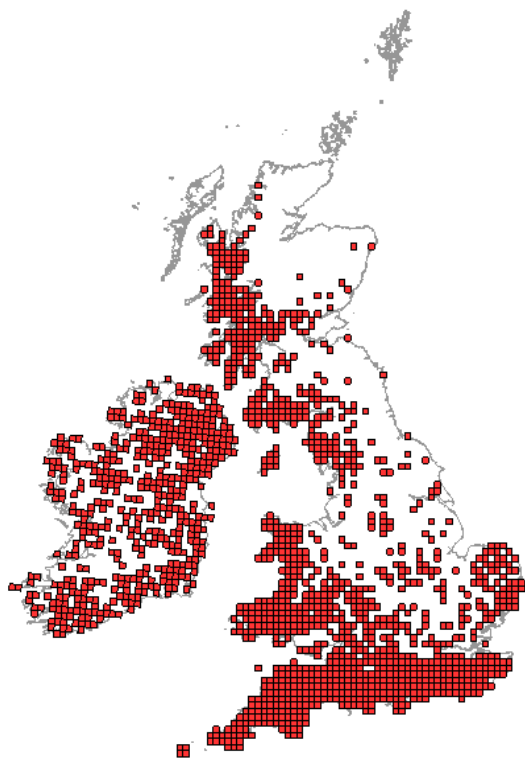
All of the above species were significantly identified as indicator species on the opposing substrata in the ISA except *U. filipendula* (Tables 4.8 and 4.9), and all except *A. punctata* have previously been described as indicators by van Herk, (1999). It is likely in some instances (e.g. *A. punctata* and *U. filipendula* only recorded at four sites on trunks and branches respectively) that low sample size in the ISA may have influenced the results. Consequently, all of these species were accepted as indicators for both trunks and branches.

#### **4.8.3 Comparison of ISA with Established Lists**

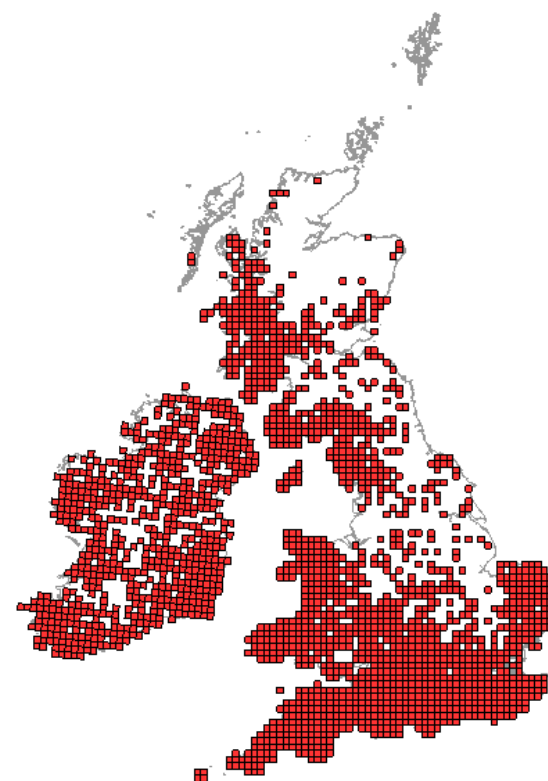
The indicator species identified in the ISA corresponded well with the findings of previous studies (van Herk, 1999; van Herk *et al.*, 2003<sup>a,b</sup>). However, five macrolichen species not previously considered as indicators were identified:

*Hypotrachyna revoluta*, *Parmelia sulcata*, *Punctelia subrudecta*, *Ramalina fraxinea*, and *Sphaerophorus globosus* (Tables 4.8 and 4.9). *Parmelia sulcata*, *Punctelia subrudecta* and *S. globosus* were accepted as potential indicators, whilst the remaining two were excluded because their distributions might have been primarily influenced by other environmental factors.

*H. revoluta* was excluded as an indicator because of concerns that its distribution was associated with climate (P.A. Wolseley, Pers. comm., 2011). Observations on the species distribution in 10 km square grid distribution maps (D-maps) downloaded from the National Biodiversity Network (NBN) Gateway (NBN, 2011) confirmed its predominantly southern and western distribution across the UK (Figure 4.10). Notably, climate was also the likely reason why *Flavoparmelia caperata* was not identified as an indicator, despite its inclusion as a nitrophyte in work by Wolseley *et al.* (2009). ISA classified this species as a nitrophile on the trunks, and a nitrophobe on the branches, and in neither instance was the result significant (ISA;  $IV = 9.5$ ;  $P = 0.1832$  and  $IV = 4.0$ ;  $P = 0.8306$ , respectively). The D-map for *F. caperata* (Figure 4.11) also displayed a bias towards the south and western edges of the UK similar to that of *H. revoluta*.



**Figure 4.10** 10 km square D-map of *Hypotrachyna revoluta* (Flörke) Hale 1975 across Great Britain and Ireland. Source: NBN (2011). See Appendix I for list of data contributors



**Figure 4.11** 10 km square distribution map of *Flavoparmelia caperata* (L.) Hale 1986 across Great Britain and Ireland. Source: NBN (2011). See Appendix I for list of data contributors.

*R. fraxinea* was identified as significantly nitrophilic (ISA;  $IV = 6.1$ ;  $P = 0.0118$ ) on branches in the ISA analysis (**Table 4.9**), even though it was previously classified as an intermediate species (OPAL, 2011). Despite this, the species was excluded as an indicator species on three accounts. The first was the confounding effect of exposure on the species. *R. fraxinea* displays a preference to wind and light exposed conditions, and this is validated by its presence on branches and absence on the trunks at sites surveyed in this study (**Appendix F**). Also, personal observations indicated that when present on the trunks of trees, the species displayed a bias towards the more exposed aspects of the tree bole. Second, *R. fraxinea* is very sensitive to SO<sub>2</sub> pollution (Smith *et al.*, 2009). This casts doubt on the reliability of its distribution at sites where perturbation through [SO<sub>2</sub>] pollution is likely to exist, or has previously done so. However, this does not preclude the lichen for use in future bio-monitoring schemes, should successful re-colonisation at previously [SO<sub>2</sub>] affected sites occur. Finally, the lichen was only recorded on *Quercus* trees during this survey, and is known to colonise trees with nutrient-rich bark (Smith *et al.*, 2009); thus, adding further restrictions to the practicality of its use in a wider scale bio-monitoring scheme.

#### **4.8.4 Formulation of Indicator Species Shortlist for Use in the Bio-monitoring Scheme**

Before a review of the most suitable FL<sub>AN</sub> scoring system could be undertaken (Section 5.2), a final shortlist of indicator species had to be produced that fulfilled the criteria described in Section 2.13.2. The shortlist of species indicators (Table 4.11) from those identified in Section 4.8.1 was evaluated



using the three criteria of distribution, frequency of occurrence on different substrata, and relative ease of field identification.

**Table 4.10** Shortlist of indicator species used in the assessment of the bio-monitoring scoring system. Species were selected from indicator species lists obtained from analysis in PC-Ord™ based upon UK distribution, location on tree and evenness of distribution on *Betula* and *Quercus*. *Graphis elegans* was used as a nitrophobic indicator on branches in place of *Sphaerophorus globosus*, which was absent from the substratum.

Nitrophobic	Nitrophilic
<i>Bryoria fuscescens</i> *	<i>Amandinea punctata</i>
<i>Evernia prunastri</i>	<i>Arthonia radiata</i>
<i>Graphis elegans</i> †	<i>Candelariella reflexa</i>
<i>Hypogymnia physodes</i>	<i>Lecidella elaeochroma</i>
<i>Hypogymnia tubulosa</i>	<i>Physcia adscendens/tenella</i>
<i>Ochrolechia androgyna</i>	<i>Punctelia subrudecta</i>
<i>Parmelia saxatilis</i>	<i>Xanthoria candelaria</i> s.l.
<i>Pseudevernia furfuracea</i>	<i>Xanthoria parietina</i>
<i>Sphaerophorus globosus</i> ‡	<i>Xanthoria polycarpa</i>
<i>Usnea</i> spp.	

\* Recorded on *Betula* only in survey

† Recorded on branches only

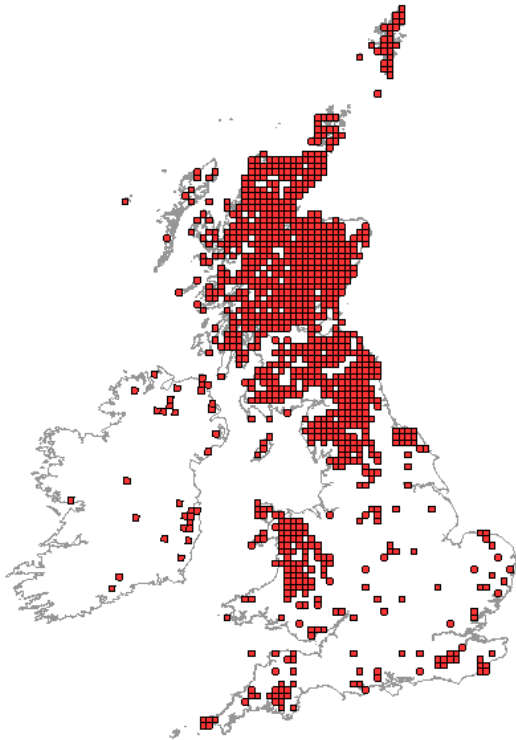
‡ Recorded on trunks only

#### 4.8.5 Distribution of Shortlisted Indicator Species

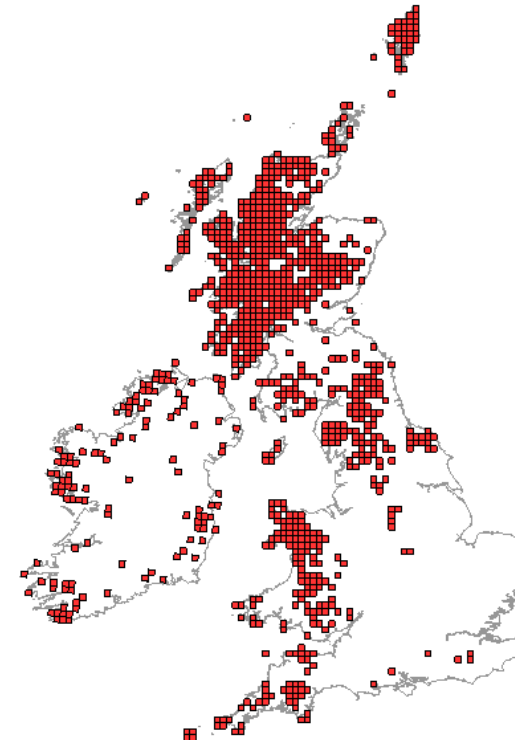
Species distributions of the shortlisted species were viewed using 10 km square D-maps downloaded from the NBN Gateway (NBN, 2011). The D-map for *Usnea subfloridana* was used as a representative for all *Usnea* spp., as it possessed the greatest distribution across the UK and overlapped areas where many of the other *Usnea* spp. were recorded. The D-maps (Appendix H) showed a very good UK-wide distribution for all of the shortlisted species except for three: the nitrophobic *Bryoria fuscescens* and *Sphaerophorus*

*globosus*, and the nitrophilic *Punctelia subrudecta* (Figures 4.12-4.14). Records of *B. fuscescens* and *S. globosus* were restricted in England to the north and far south west. The distribution of *P. subrudecta* s.l. was even more limited, being restricted to the southern end of the UK. However, a 10 km D-map of *P. subrudecta* s.s. (Figure 4.14a) displayed a wider distribution across Ireland, W. Scotland and SW England compared to *P. subrudecta* s.l. (Figure 4.14b). The additional information from this second D-map helped to expand the known distribution of the species across the UK, but major gaps still persisted throughout central and northern England and the Scottish Highlands.

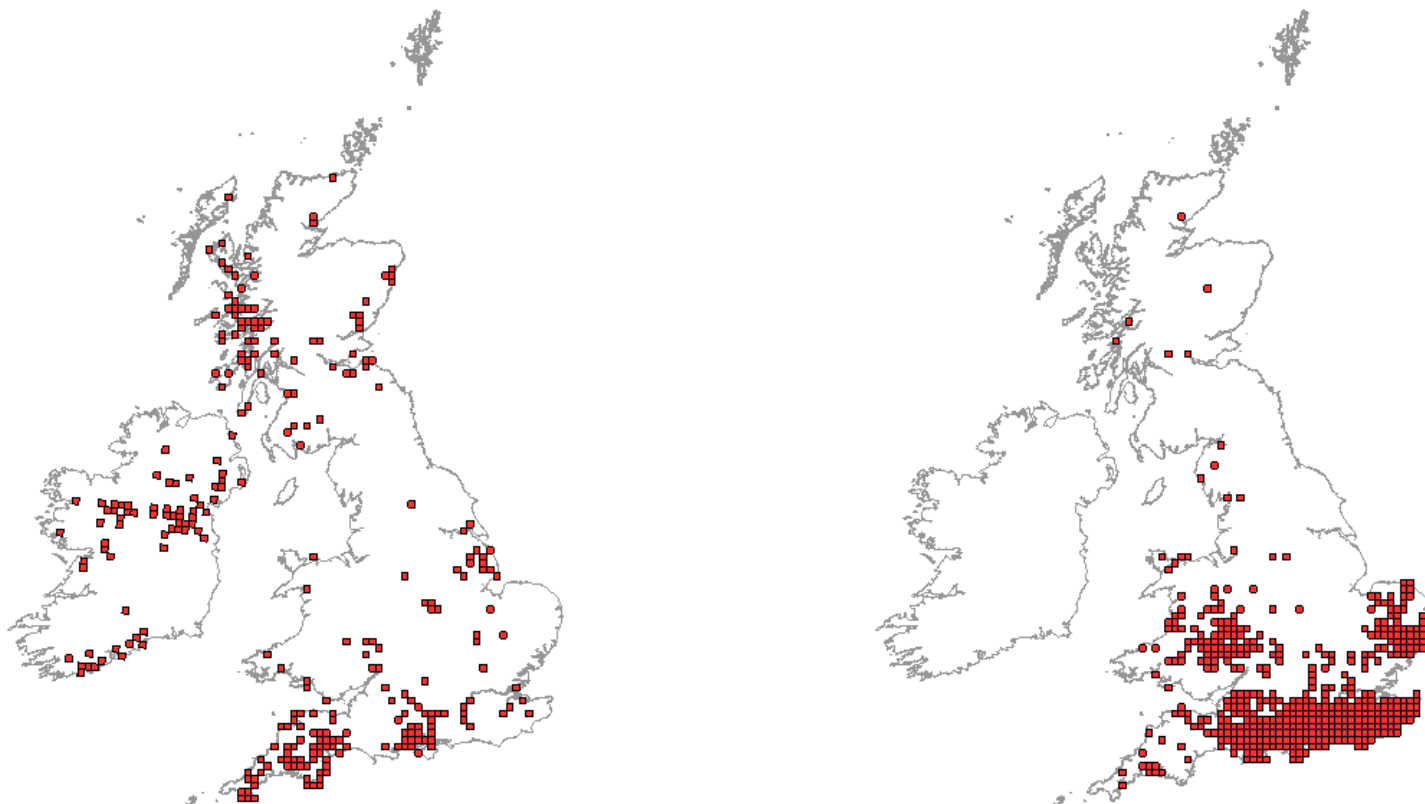
The D-maps of these three species shared a similar pattern as five other shortlisted indicators (*Pseudevernia fufuracea* s.l., *Arthonia radiata*, *Lecidella elaechroma*, *Ochrolechia androgyna* and *Usnea* spp.) whereby gaps appeared in the distributions across the Midlands of England (Appendix H). The gaps in species presence in this region is likely due to the historical perturbation caused by SO<sub>2</sub> pollution and possibly reflects a slower re-colonisation rate for these species. Despite this the remaining ten indicators (four nitrophobic and six nitrophilic) have all been recorded in this part of the UK, and thus, provide indicator species coverage for this region. The disparity in the number of available indicator species between this temporally perturbed region and the rest of the UK lends further credence to the establishment of an un-weighted bio-monitoring scoring system, as this would not bias the results in favour of the more undisturbed, species rich areas of the UK.



**Figure 4.12** 10 km square distribution map of *Bryoria fuscescens* (Gyeln.) Brodo & D. Hawksworth 1977 across Great Britain and Ireland. Source: NBN (2011). See Appendix I for list of data contributors.



**Figure 4.13** 10 km square distribution map of *Sphaerophorus globosus* (Huds.) Vain 1903 across Great Britain and Ireland. Source: NBN (2011). See Appendix I for list of data contributors.



**Figure 4.14** 10 km square distribution map of (a) *P. subrudecta* s.s. (Nyl.) Krog 1982 and (b) *Punctelia subrudecta* s.l. across Great Britain and Ireland. Source: NBN (2011). See Appendix I for list of data contributors.

#### **4.8.6 Frequency of Occurrence**

The percentage frequency of occurrence on phorophytes and substratum was well balanced for most of the shortlisted lichens, however, some did display a bias. *Bryoria fuscescens* was found exclusively on *Betula*. Also, although specimens of the nitrophilic *Amandinea punctata* and *Punctelia subrudecta* were found on both phorophytes, they both showed a bias towards *Quercus* on the branches where they were specifically identified as being significant indicator species (Table 4.9). One further observation was that the nitrophobic *Sphaerophorus globosus* was only recorded on tree boles (Appendix F).

#### **4.8.7 Ease of Identification**

This criterion was introduced because of the fundamental need to use species that were relatively easy to identify in the field by non-experts. Any possible identification problems for species need to be rectified before their inclusion into the bio-monitoring scheme. Many lichen species can be accurately identified by basic morphological or chemical techniques, and often a combination of these factors is required. Microscopic investigations are also often required; however, the practicality of implementing this approach in the bio-monitoring scheme is questionable given the uncertainties on the availability of microscope equipment for individuals. Therefore, the key characteristics differentiating superficially similar species should be based on the macroscopic level, using morphological and chemical characteristics as much as possible to enable a more rapid field evaluation.

All of the shortlisted indicator species proposed for use in the bio-monitoring scheme (Table 4.10), and used in evaluation of the scoring system (Chapter 5), had at least one other species associated with them with which they could be misidentified (Appendix J). In some instances similarity between species was not deemed to have any discernable effect on the scoring system. For example, *X. candelaria* and *X. ucrainica* were already aggregated under *X. candelaria* s.l. in the indicator species list. Similarly, confusion between *Bryoria fuscescens* and other species of *Bryoria* spp. is possible, especially *B. subcana*. But *Bryoria* could, in theory, be grouped together at the generic level in the same manner as *Usnea* spp. given the preponderance for the species in the genus to be a calcifuge (Smith *et al.*, 2009). In a few other cases, the possibility of misidentification of certain indicator species with other lichens was perceived to be with other similar indicators, e.g. the nitrophobic indicators *Hypogymnia physodes* and *H. tubulosa*. However, the large proportion of species that were morphologically similar to each indicator species were not themselves indicators, so determination of the species was an imperative.

Macroscopic morphological characteristics to identify indicator species from other similar looking species existed to varying degrees in all but three instances (Appendix J). In two of these cases, chemical spot tests were identified as the simplest means of separating the two species: i.e. a thallus K spot test to separate *Amandinea punctata* from *Buellia disciformis*, and a soralia C spot test to separate *Ochrolechia androgyna* from *Lecanora alboflavida* (Appendix J). *Amandinea punctata* and *Buellia schaeferi* can only be confidently separated by means of microscopic examination. However,

recordings of *B. schaereri* suggest it is restricted to upland regions of Scotland and Wales, so any possible complications arising between these two species would be confined to these areas (Appendix J).

## **4.9 Discussion**

### **4.9.1 Surveyed Tree Distance from Monitoring Stations**

Most trees sampled were within the target 1 km radius of the monitoring station for each site. The two sites where mean tree distance exceeded 1 km (Dunalastair Estate and Rannoch Forest) were associated with the lowest [NH<sub>3</sub>] values among the 28 survey sites and no point sources were known to exist between the monitoring station and surveyed trees. Therefore, it is probable that the [NH<sub>3</sub>] values recorded at the stations were an accurate estimate of mean values to which the trees were exposed.

### **4.9.2 Bark Topography**

The similarity of mean bark topography between the two tree genera implied that from a microclimatic viewpoint the trees were broadly comparable. However, *Betula* and *Quercus* bark differ in a number of other physicochemical ways, such as water holding capacity (Levia & Herwitz, 2005), level of exfoliation (Ahti, 1977) and types and quantities of tannins, betulins and resins (Barkman, 1958<sup>b</sup>; Brodo, 1974). The negative relationship between Bt and gbh on *Quercus* trees supported the hypothesis that a reduction in the Bt score (i.e. increase in furrow to ridge ratio) could be indicative of tree age. However, as only a weak relationship was observed between these two measurements and no accurate quantitative measure of tree age undertaken,

such as core sampling, (Ellis & Coppins, 2006), a definitive conclusion cannot be drawn. Bark topography has previously been shown to be important for both micro- and macrolichens (Ellis & Coppins, 2007; Lamit *et al.*, 2011); therefore the indicators selected should exclude lichens that have known associations with bark crevices, such as *Chaenotheca furfuracea* (Smith *et al.*, 2009) to ensure it has no bearing on the FL<sub>AN</sub> score.

#### **4.9.3            Assessment of Branch Length**

Mean branch length was greater in the 6-10 year class (56.34 cm) than the 1-5 year class (40.13 cm) across the 20 sites where measurements were made, and consistently longer than the 1-5 year age classes within each site (Figure 4.3). There was also considerable inter-site variation in branch length probably due to a number of factors, such as tree age and health, environmental stress and nutrient availability. It was consequently concluded that for the purposes of a bio-monitoring scheme that required segregating branches into sections it would be equally practical, and less time consuming, to separate the branches into sections of equal length (e.g. 0-50 cm, 51-100 cm and 100-150 cm) as to count the growth scars for age-based branch segregation.

#### **4.9.4            Evaluation of Sampling**

Assessment of the SR accumulation curves identified that surveying five replicate trunks for both *Betula* and *Quercus* was insufficient to confidently determine SR at a site (Appendix D). On the other hand, a baseline of 10 replicate branches located the majority of the more common epiphytic species,



despite the SR accumulation curves not becoming asymptotic at some locations (Appendix E).

#### **4.9.5 Importance of Species Richness in Indicator Species Selection**

The significantly smaller number of bryophytes recorded during the survey is probably an artefact of the survey technique. Selection of exposed trees likely reduced the number of bryophytes (and to a lesser extent, lichens) because free-standing trees are exposed to lower humidity levels (Sonnleitner *et al.*, 2009). Additionally, the omission from the survey of the lower section of the trunks (Section 2.8) excluded the recording of pleurocarpous bryophytes at several sites where they were restricted to the base of the tree boles. This was a potentially beneficial exclusion because establishment of mat forming pleurocarpous species such as *Hypnum* spp., *Isothecium* spp. and *Thuidium* spp. can result in competitive exclusion of many lichen species. Given the larger ratio of lichen species to bryophytes in the UK, and the theoretically greater number of identifiable indicator species, the focus of the bio-monitoring scheme remained with lichen species.

Total microlichen SR was greater than that for macrolichens. One reason for this was that many of the macrolichens recorded were present on both the trunks and branches. The main exceptions to this were the largely trunk restricted *Cladonia* spp. and branch restricted *Melanohalea* spp. In contrast, there was a greater segregation between branches and trunks amongst microlichens, with species such as *Arthonia spadicea*, *Calicium viride*,

*Chrysothrix candelaris*, *Lecanactis abietina*, *Schismatomma decolourans* and *S. niveum* being recorded exclusively on trunks; and others such as *Arthopyrena analepta*, *A. punctiformis*, *Cyrtidula quercus*, *Phaeographis dendritica* and *Rinodina sophodes* only on branches. This is exemplified by the complete disparity in microlichen species composition observed on the trunks and branches at Wood of Cree.

Reasons for the greater substratum specificity of microlichens could be linked to numerous factors. These include temporally-driven dependant variables such as bark topography and the colonisation rate of individual species (Ellis & Coppins, 2007; Topham, 1977), and also autogenic factors such as facilitation, parasitism and competitive exclusion (Honegger, 2008; John & Dale, 1995). The large number of microlichens recorded emphasises the importance of their inclusion as bio-indicators. However, selection of specific microlichen species in the bio-monitoring scheme needs careful consideration for two reasons. First, some common microlichens are unevenly distributed between branches and trunks; and second, identification of microlichens is often more difficult than that of macrolichens, especially in the field.

#### **4.9.6 Evaluation of NMDS Ordination Analysis of Survey Data**

The NMDS ordination results and subsequent plots were reliable according to Mantel Test results on the five replicate ordination analyses (Section 2.13.1). The ordination supported the correlation analyses of atmospheric chemistry variables, in particular the relationship between  $[SO_2]$  and  $[NO_2]$  and the relationship between  $[NH_3]$  and  $[NH_4^+]$ . Two further relationships were

identified, one between  $[\text{NH}_3]$  and pH, which concurs with current knowledge (de Bakker, 1989; van Herk, 2001), and the other between OI and base cations. On a broader scale, two further items of note from the ordination were the polarisation of all the atmospheric chemistry variables (except base cations) away from the OI gradient and the segregation of the phorophyte genera in the ordination.

The negative relationship between many of the atmospheric chemistry variables and the OI could be either an artefact of land management, e.g. different grazing regimes under differing climate conditions. Another explanation might be the presence of a direct effect of a climatic factor such as precipitation, on atmospheric chemistry concentrations.

The spread of the different phorophyte genera in relation to the OI in the ordination (Figures 4.7 and 4.9) implies that the disjunction of epiphytic species composition on *Betula* and *Quercus* could be due to an artefact of climate rather than one of a phorophyte influence. Although this was reflected to some degree at some survey sites, there was nonetheless overlap in climatic conditions between the distributions of *Betula* and *Quercus*. A three dimensional evaluation of the spread of phorophytes in the ordination supported this by showing that they were not constrained entirely by climate (as defined by the OI). Further confirmation came from the five sites where both tree genera were surveyed, which had a range of climatic conditions. These five sites provide a useful framework with which to investigate the potential influence of phorophytes on community composition, but also the

effect of phorophyte on the bio-monitoring scoring system (Sections 5.5 and 5.6.4).

Another matter supporting the natural influence of phorophyte species on epiphytic community composition is the preference that some lichen species show to certain tree species. In the present study *Leptorhaphis epidermis*, *Bryoria fuscescens* and *Tuckermanopsis chlorophylla* were found exclusively on *Betula* and *Cyrtidula quercus* and *Pyrrrhospora quercea* found only on *Quercus* (Appendix F). This agrees with the documented host preferences of the species: *L. epidermis* is specific to *Betula* (Dobson, 2005; Smith *et al.*, 2009), *B. fuscescens* and *T. chlorophylla* occur predominantly on *Betula* and conifer tree species (Dobson, 2005), whilst *C. quercus* and *P. quercea* predominantly occur on *Quercus* and *Corylus* trees (Smith *et al.*, 2009).

#### **4.9.7 Identification of Indicator Species**

Several species previously reported to be indicator species for [NH<sub>3</sub>] or N-enrichment (van Herk, 1999; van Herk *et al.*, 2003<sup>a,b</sup>; Wolseley, 2005) were excluded from both the NMDS ordination and ISA analyses during the short-listing process (Section 4.6) because of the limited number of sites they were recorded at. These were: *Candelariella vitellina*, *C. xanthostigma*, *Diploica canescens*, *Hypocenomyce scalaris*, *Imshaugia aleurites*, *Lecanora dispersa* s.l., *Ochrolechia microstictoides*, *Physcia leptalea* and *Usnea rubicunda*. In addition to these species, a number of established indicator species were not recorded at all during the survey, including *Phaeophyscia orbicularis*, *Hyperphyscia adglutinata* and *Parmelina* spp. Accordingly, the

indicator species list formulated in this thesis is an alternative grouping and does not necessarily supplant lists produced by other workers.

#### **4.9.7.1 Preliminary Identification of Indicator Species Using NMDS**

Species groupings observed in the ordination analyses provide a good baseline from which to identify indicator species, and the reliability of the ordination is endorsed by the strong overlap in the species identified with those currently listed as indicators. Over 66% of the macrolichens identified as possible indicators on the basis of ordination results (Section 4.6.2) were previously listed as such (van Herk, 1999; Wolseley, 2005; Wolseley *et al.* 2009). The ordination analyses also brought to light the need to consider three important matters: the potential influence of climate on the legitimacy of using certain species as bio-indicators; the potential influence of tree species on epiphyte community composition, and subsequently indicator species presence on certain trees; and the need to consider the relative distribution of species on trees when the final draft of indicator species is formulated.

Indicator species found on both tree trunks and branches are the ideal candidates for inclusion in a bio-monitoring method, providing that they are easy to identify. However, the number of such species was likely to be insufficient and may need supporting by inclusion of indicator species that were identified for either trunks or branches only. It is important to note that species identified as indicators on only one substratum type during this study were not automatically excluded. The reason for this was that in some instances species were recorded on both substrata, but they were only included

in the ordination analysis for one substratum type. One example of this was *Arthonia radiata*, which was identified as a potential indicator for branches, but not for trunks. This species was excluded from the trunk ordination because it was only recorded on trunks at two sites (Appendix F). It was not assumed acceptable to include automatically all such species as indicators on both substrata. For example, *Cladonia polydactyla* and *Ochrolechia androgyna* were considered potential nitrophobic indicator species on trunks in the ordination analyses, but not for branches, for the same reason as described for *A. radiata* above. However, the presence of these two species on branches was restricted to the oldest parts of the surveyed branches. Being later colonisers (Topham, 1977) would fundamentally undermine their use as bio-indicator species on branches. Hence, early colonising indicator species should be given more importance in branch surveys. It was therefore concluded that lichens found on, and identified as indicators for, both substrata on both tree genera were selected in the first instance; and those recorded as either trunk or branch specific used thereafter.

#### **4.9.7.2 Identification of Indicator Species for [NH<sub>3</sub>] using ISA**

Epiphytes identified as either nitrophobic or nitrophilic indicator species in the ISA largely reflected that of established work (van Herk, 1999; Wolseley, 2005; Wolseley *et al.* 2009) and the NMDS ordination analysis. Only two notable discrepancies were observed: *Chaenotheca ferruginea* and *Lepraria incana* (Section 4.8.1). The expansion of this study to include microlichens enabled a more holistic view of potential UK indicator species. Unfortunately, possible complications in field identification made it impractical to use some of

these lichens, which included several species of *Lecanora* (Tables 4.8 and 4.9). If these species were included they would have been most influential on the branches, given their typically greater abundance on the younger substratum (Appendix F). Since tree branches would not be influenced by the temporal effects of past SO<sub>2</sub> perturbation as much as trunks, then the use of these lichens on the branches would theoretically provide a more accurate estimation of [NH<sub>3</sub>] at sites. Although it was not feasible in the context of the construction of this bio-monitoring scheme to include them, consideration should be given to their future use.

The possibility of species misidentification by users of the bio-monitoring scheme is an issue that requires addressing. Key characteristics that can be used in the field to distinguish between an indicator lichen and morphologically similar species were provided for all indicators (Appendix J). In all but one instance (identifying *Amandinea punctata* from *Buellia schaererii*), field-based techniques can be used to confirm the presence of the shortlisted indicator species. In many of these cases it was possible to find characteristics based on either morphology or spot testing with C, which could be undertaken simply by any user of the bio-monitoring scheme with access to a hand lens and household bleach. In just four instances was it necessary to include K spot testing, and in only one of these (identifying *Amandinea punctata* from *Buellia disciformis*) was the spot testing the only way to separate the species in the field. The distributions of morphologically similar species also proved beneficial in establishing specific regions where possible

problems would be greatest, e.g. *B. schaereri* in upland Scotland and Wales and *B. disciformis* in the north and west of Britain.

Any misidentification is likely to have deleterious effects on the four FL<sub>AN</sub> scoring systems tested in Chapter 5, and subsequently the accuracy of the bio-monitoring scheme. No potential confusion between indicator species from the two indicator classes in the shortlist was identified (Appendix J), which limited the effects of misidentification somewhat. However, this only reduced, and did not eradicate the problem. One example exists whereby misidentification could arise between a shortlisted nitrophile (*Lecidella elaechoroma*) and an unlisted species (*Fuscidea lighfootii*) identified as a potential nitrophobe (Appendix J; Table 4.9). A slightly higher risk existed in the misidentification of species within each indicator class, e.g. the two nitrophobes *Hypogymnia physodes* and *H. tubulosa*. This was not predicted to be problematic when calculating a score based on presence and absence of indicators at the indicator class level (i.e. nitrophile or nitrophobe) rather than the indicator species level. However, any scoring technique that implemented a weighting for SR or diversity would potentially be influenced to varying degrees. Non-indicator species were the largest group of morphologically similar lichens to the indicator species, and although the effect of misidentification of these is not as great as it would be between nitrophobic and nitrophilic species, it would nonetheless affect all four scoring systems. Thus, the need for clear identification of the shortlisted indicator species is critical.



## **Chapter 5. Establishment and Evaluation of a Scoring System**

### **5.1 Introduction**

It has been reported by Motiejūnaitė (2007) that the best indicators of community characteristics are based on species richness (SR), presence/absence and abundance. Several methods that use lichens as bio-monitors of N pollution in Europe have been formulated that are based on, or weighted for, abundance. These include the Acidofiele Indicate Waarde (AIW) and Nitrofiele Indicate Waarde (NIW) (van Herk, 1999), and the Lichen Acidophyte Nitrophyte ( $L_{AN}$ ) methods (Sutton *et al.*, 2004; Wolseley *et al.*, 2009). The abundance-based weighting system used in the AIW and NIW scoring systems factors in both number of thalli and the area covered by each species. Use of this method was not deemed practical for rapid appraisal by non-specialists due to the time-consuming nature of the surveying technique and potential bias afforded towards species that propagate vegetatively down tree boles.

The  $L_{AN}$  method, as used by Wolseley *et al.* (2009) is a more time efficient method that also implements an abundance-based weighting measure. Although proven effective in its application with the indicator species it utilises, this technique, as with that of van Herk (1999), would favour a few larger spreading lichen species. Therefore, an abundance-based weighting technique was not considered in the interests of this study because of the greater bias towards macrolichens compared to microlichens resulting from the

typically smaller size of the latter. Instead a Frequency-based Lichen Acidophyte Nitrophyte (FL<sub>AN</sub>) was applied based on presence/absence data. A weighting method based on SR was also investigated to determine whether the number of different indicator species recorded would produce a stronger relationship with [NH<sub>3</sub>] values.

Following assessment of SR weighted and unweighted FL<sub>AN</sub> techniques, identification of confounding variables was undertaken to enable their inclusion into a regression equation. Significant correlates were then utilised in a regression model for estimated [NH<sub>3</sub>] values. Air concentrations and deposition of other N forms and the historically influential S compounds were evaluated together with those for climate and bark pH, which have both previously been shown to modify lichen community composition and bio-monitoring results (Ellis & Coppins, 2006; Ellis *et al.*, 2007; Larsen *et al.*, 2007; Mežaka *et al.*, 2008; van Dobben & ter Braak, 1998; van Herk, 2001; Wolseley *et al.*, 2005, 2006<sup>a</sup>).

A final consideration of the efficacy of the FL<sub>AN</sub> system was to determine the consistency of results between different phorophyte species. There is a general acceptance that phorophyte type is of significant importance to cryptogam community composition (Culberson, 1955; Hale, 1955; Kuusinen, 1996; Martin, 1938; Spier *et al.*, 2010), and this matter has been factored into a number of studies investigating N effects (Frati *et al.*, 2007; van Herk, 1999). Given the natural variation in bark pH between tree species (Barkman, 1958<sup>a</sup>), which was a potential confounding factor in this study, the trees surveyed were

restricted to the two acid-barked genera *Betula* and *Quercus* (see Section 2.8). These two tree genera show a difference in geographical distribution, with *Betula* dominating in colder, wetter climates of the north and north western areas of the UK where *Quercus* may be absent (Atkinson, 1992; Jones, 1959). However, it was previously concluded that sufficient heterogeneity in the sampling existed in the survey to counter this effect (Section 4.9.6). To further strengthen comparisons between phorophytes, analyses of FL<sub>AN</sub> scores and bark pH were undertaken for the five survey sites where both *Betula* and *Quercus* were surveyed.

## **5.2 Methods**

### **5.2.1 Assessment of FL<sub>AN</sub> Scoring System**

The effect of weighting L<sub>AN</sub> scores for SR was investigated by comparing the goodness of fit between [NH<sub>3</sub>] and the four FL<sub>AN</sub> variants: Non-accumulative Un-factored FL<sub>AN</sub> NUFL<sub>AN</sub>, Non-accumulative Factored FL<sub>AN</sub> NFFL<sub>AN</sub>, Accumulative Un-factored FL<sub>AN</sub> AUFL<sub>AN</sub> and Accumulative Factored FL<sub>AN</sub> AFFL<sub>AN</sub> (calculated as described in Section 2.13.3 and Appendix K). The NUFL<sub>AN</sub> was the only scoring system that was not SR-weighted; this produced a final indicator score based on the frequency of each indicator class (i.e. nitrophobes and nitrophiles) on surveyed trees rather than at the individual indicator species level. Hence, a score of 0-3 was obtained for each indicator class at each site, as calculated from the mean of the trees surveyed. The SR weighting for the NFFL<sub>AN</sub> was obtained by multiplying the frequency of each indicator class on each tree by the number of different indicator species of each respective indicator class recorded on it, before calculating mean tree score for

each site, producing a range of scores from 0-27. SR weighting in the AUFL<sub>AN</sub> was obtained by summing the frequencies of the each indicator species in each class on each tree. Therefore, this approach provided the same scoring range as the NFFL<sub>AN</sub>, but unlike the NFFL<sub>AN</sub>, it considered the individual frequencies of each indicator species on each aspect of the tree. The AFFL<sub>AN</sub> was the most heavily SR-weighted technique, using a hybrid approach that utilised the additive SR weighting of the AUFL<sub>AN</sub> and the product weighting of the NFFL<sub>AN</sub>, hence, producing a scoring range from 0-243. Relationships between measured [NH<sub>3</sub>] values and the four FL<sub>AN</sub> variants were examined. The FL<sub>AN</sub> technique displaying the strongest relationship with [NH<sub>3</sub>] was accepted as being the most robust technique for use in the bio-monitoring scheme, and was also selected for all further analyses in this Chapter.

## **5.2.2 Identification of Confounding Environmental Variables**

Relationships between the optimum FL<sub>AN</sub> and environmental variables other than [NH<sub>3</sub>] were examined in order to identify potential confounding factors. This was achieved by examining correlations between FL<sub>AN</sub> scores (both trunk and branch) and climate variables, atmospheric chemistry and substratum pH at each survey site. In the case of climate, FL<sub>AN</sub> scores were plotted against four climate variables: oceanicity index (OI), annual rain days > 1mm (RD > 1 mm), annual rain days > 10mm (RD > 10 mm) and mean annual precipitation (PPN). For the investigation into atmospheric chemistry, both concentration and deposition variables were evaluated. Concentration values were obtained from a mix of modelled and measured data sources (see Table 3.4) and deposition derived purely from a model. Fine Resolution

Atmospheric Multi-pollutant Exchange (FRAME) model datasets at the 5 km resolution were used in all instances, with the exception of [NO<sub>2</sub>], where 1 km resolution data were used.

### 5.2.3 Formulation of an Optimum Model for the NUFL<sub>AN</sub> Scoring System

Ammonia concentration and the most significant confounding variables were used to build an optimum model for estimating FL<sub>AN</sub> scores. Ammonia was entered into the model first. Other variables were entered in order of decreasing significance, as determined by the analysis of confounding environmental variables. To provide standardisation in the calculation of [NH<sub>3</sub>] and other N compounds, ratios between compounds were calculated based on the number of micro moles (#μM) of each, as calculated from the molarity ( $c_i$ ), which equated to the concentration in air (μg m<sup>-3</sup>), and the molecular mass (M) of each compound (Eq. 5.1). The robustness of the regression models established for trunks and branches was tested by plotting the predicted (modelled) FL<sub>AN</sub> scores, based on the regression models, against measured FL<sub>AN</sub> scores from the survey sites.

$$\# \mu M = c_i / M \quad [\text{Eq. 5.1}]$$

### 5.2.4 Influence of Phorophyte Type on NUFL<sub>AN</sub> Scores

The effect of phorophyte type on FL<sub>AN</sub> scores was first assessed by comparing [NH<sub>3</sub>]/FL<sub>AN</sub> relationships for *Betula* and *Quercus*. This was undertaken both for trees surveyed across all the sites and for the five sites where both *Betula*

and *Quercus* were measured. Finally, a multiple linear regression was undertaken between trunk bark pH, [NH<sub>3</sub>] and the potentially confounding factor of tree age, as represented by bark topography (Bt) and tree girth at breast height (gbh).

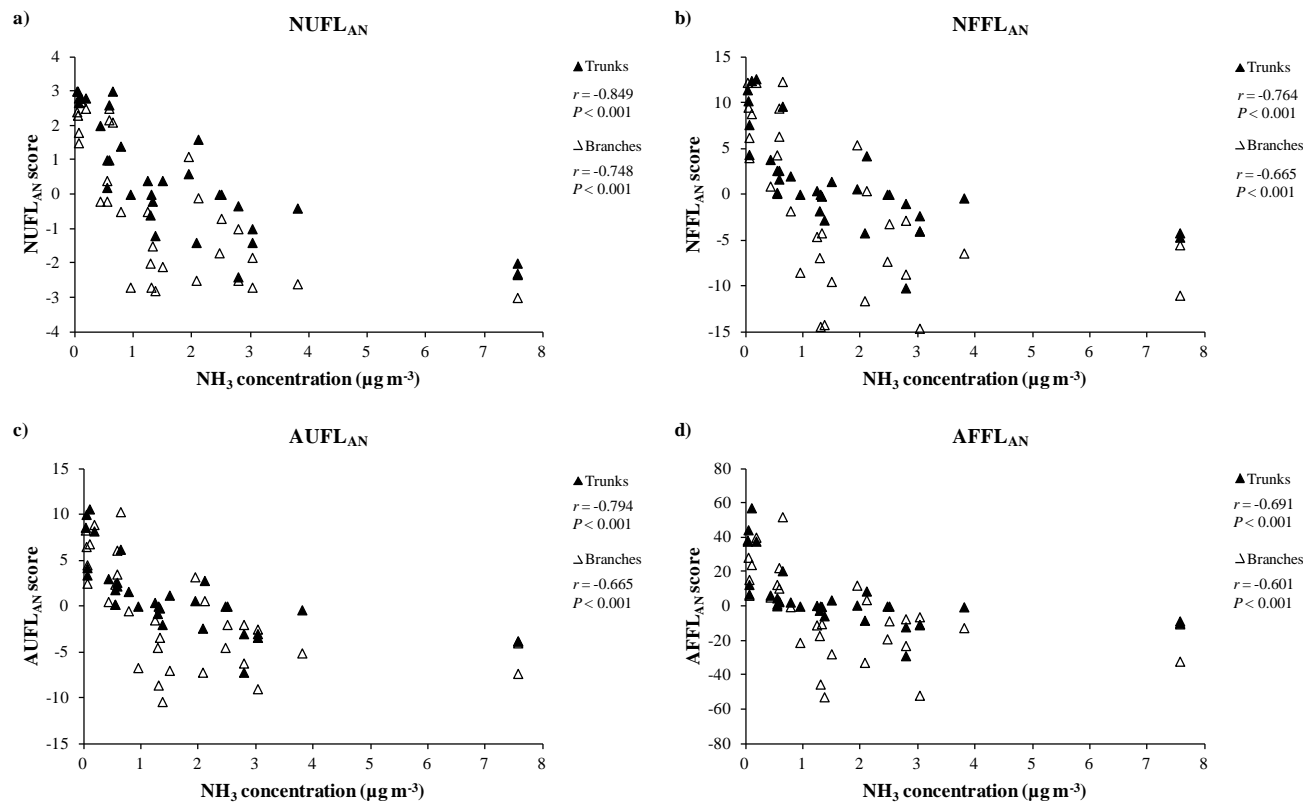
### **5.2.5 Statistical Analysis**

Relationships between FL<sub>AN</sub> methods and environmental and climatic variables were analysed using Pearson's Product Moment Correlation Co-efficient PPMCC and Spearman's Rank Correlation SRC respectively. Non-parametric SRC analysis was undertaken as normalisation of some climate datasets was not possible using standard practices. Significant differences in bark pH between the tree genera were investigated using a one-way ANOVA. Where appropriate, data were square root or Box-Cox transformed to obtain normality. Linear regression analysis was used to develop the optimum model, using square root transformed [N] data to ensure a normal distribution of these datasets.

## **5.3 Results**

### **5.3.1 Assessment of FL<sub>AN</sub> Scoring System**

Scores for all FL<sub>AN</sub> variants showed strong negative correlations with [NH<sub>3</sub>] for both the trunk and branch surveys with *r* values decreasing in the sequence: NUFL<sub>AN</sub> > AUFL<sub>AN</sub> > NFFL<sub>AN</sub> > AFFL<sub>AN</sub> (Figure 5.1). Consequently, the un-weighted NUFL<sub>AN</sub> was accepted as the scoring method of choice to undertake the remaining analysis in this Chapter.



**Figure 5.1** Relationships between measured atmospheric  $\text{NH}_3$  concentrations and  $L_{\text{AN}}$  scores at 32 survey sites in the British Isles. Four  $L_{\text{AN}}$  variations were assessed: a)  $\text{NUFL}_{\text{AN}}$ , b)  $\text{NFFL}_{\text{AN}}$ , c)  $\text{AUFL}_{\text{AN}}$  and d)  $\text{AFFL}_{\text{AN}}$ . All  $r$  values were calculated using square root transformed  $[\text{NH}_3]$  values in a PPMCC analysis.

### **5.3.2 Identification of Confounding Environmental Variables**

#### **5.3.2.1 Relationships between NUFL<sub>AN</sub> Scores, Climate and Land Use**

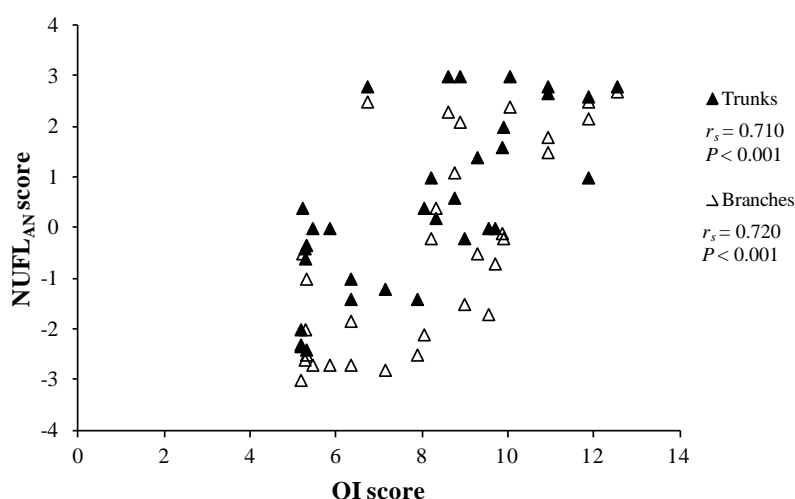
SRC analysis showed strong positive relationships between the OI and both trunk and branch NUFL<sub>AN</sub> scores (Figure 5.2). This fits the pattern observed in the ordination that drew the OI in the opposing direction to [NH<sub>3</sub>] (Figures 4.6 and 4.8), which was negatively correlated with the FL<sub>AN</sub> scores (Figure 5.1). Assessments of the three rainfall measurements gave similar results, but a slightly stronger relationship was observed between NUFL<sub>AN</sub> scores and RD > 10 mm for trunks (SRC;  $n = 32$ ;  $r_s = 0.789$ ) and RD > 1 mm (SRC;  $n = 32$ ;  $r_s = 0.747$ ) for branches. As RD > 1 mm showed the strongest general relationship with NUFL<sub>AN</sub> scores for both trunks and branches, this was used as the climatic variable in all future analyses throughout this Chapter.

The relationship between NUFL<sub>AN</sub> and the above mentioned climatic variables raises concerns that one may exist between [NH<sub>3</sub>] and climate as a result of land use, particularly as intensive agriculture practices are more prevalent in the south east of the UK. Although the form of the landscape may play a role in land use (e.g. the heavily contoured, thin soiled ground at Glen Nant, compared to the flat deeper, more nutrient-rich soils surrounding Hinderclay Fen), climate can also be influential.

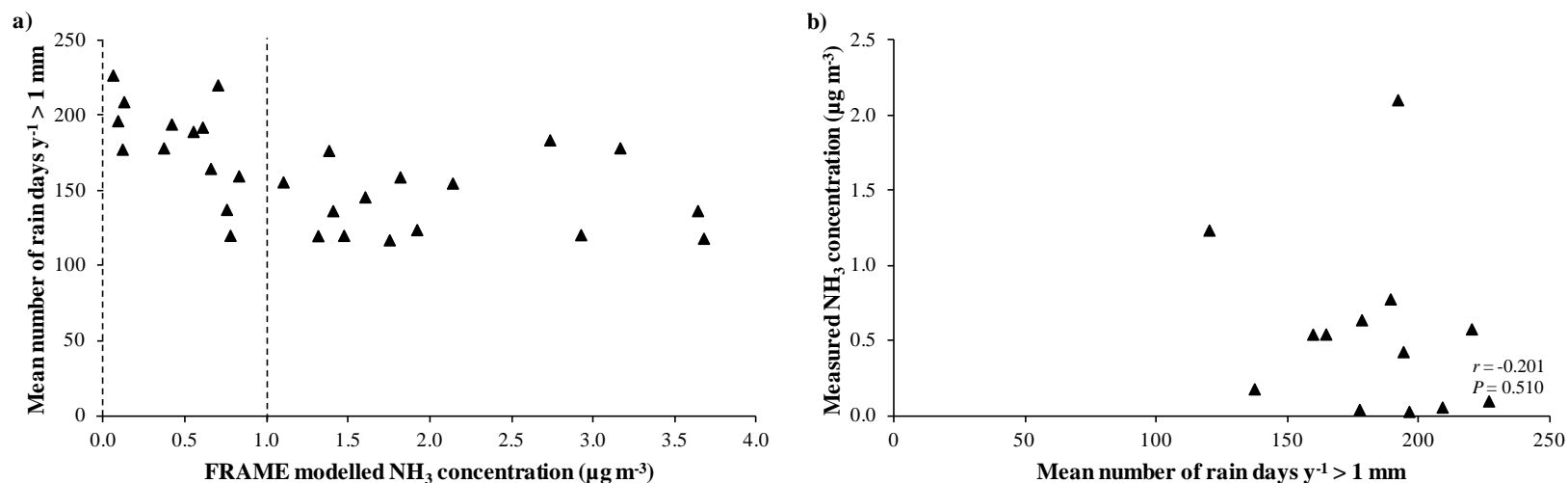
To identify whether land use, and subsequently [NH<sub>3</sub>], across the survey sites showed any link with climate, the relationship between measured [NH<sub>3</sub>] and annual RD > 1 mm was examined for a subset of the survey sites. Sites were



selected from an initial plot of FRAME modelled data against  $RD > 1 \text{ mm}$  (Figure 5.3). A total of 13 sites were selected with  $[\text{NH}_3]$  values  $< 1 \mu\text{g m}^{-3}$  covering a range of  $RD > 1 \text{ mm}$  values from 120 to 227. PPMCC analysis of this subset of sites showed no correlation between  $RD > 1\text{mm}$  and  $[\text{NH}_3]$ . The lack of a significant negative correlation in the PPMCC analysis between  $[\text{NH}_3]$  and climate (Figure 5.3b) suggests that no link existed between land use and climate at the surveyed sites.



**Figure 5.2** Relationship between climate (as represented by the OI score) and NUFL<sub>AN</sub> on trunks and branches at the 28 survey sites. Analysis using a Spearman's Rank Correlation showed a strong positive relationship between the two variables on both substrata.

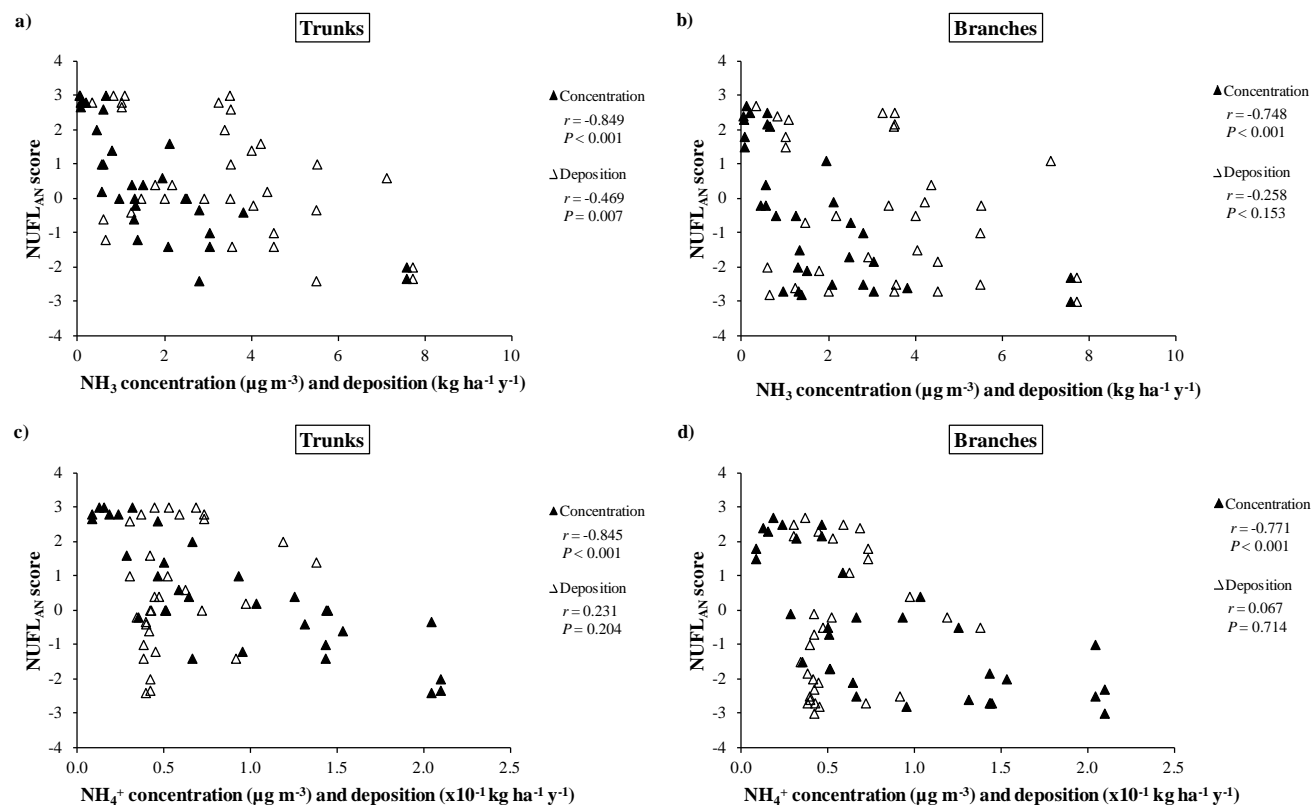


**Figure 5.3** Relationships between  $[\text{NH}_3]$  and mean number of rain days  $> 1 \text{ mm}$  using a) FRAME modelled  $1 \text{ km}^2$   $[\text{NH}_3]$  values, and b) measured  $[\text{NH}_3]$  values. The values plotted in b) were obtained from sites with FRAME modelled  $[\text{NH}_3]$  values  $< 1 \mu\text{g m}^{-3}$  (i.e. left of the dotted line in a)). A PPMCC correlation analysis on the square root transformed data did not produce a significant relationship between the measured mean annual  $[\text{NH}_3]$  and climate (as defined by rain days).

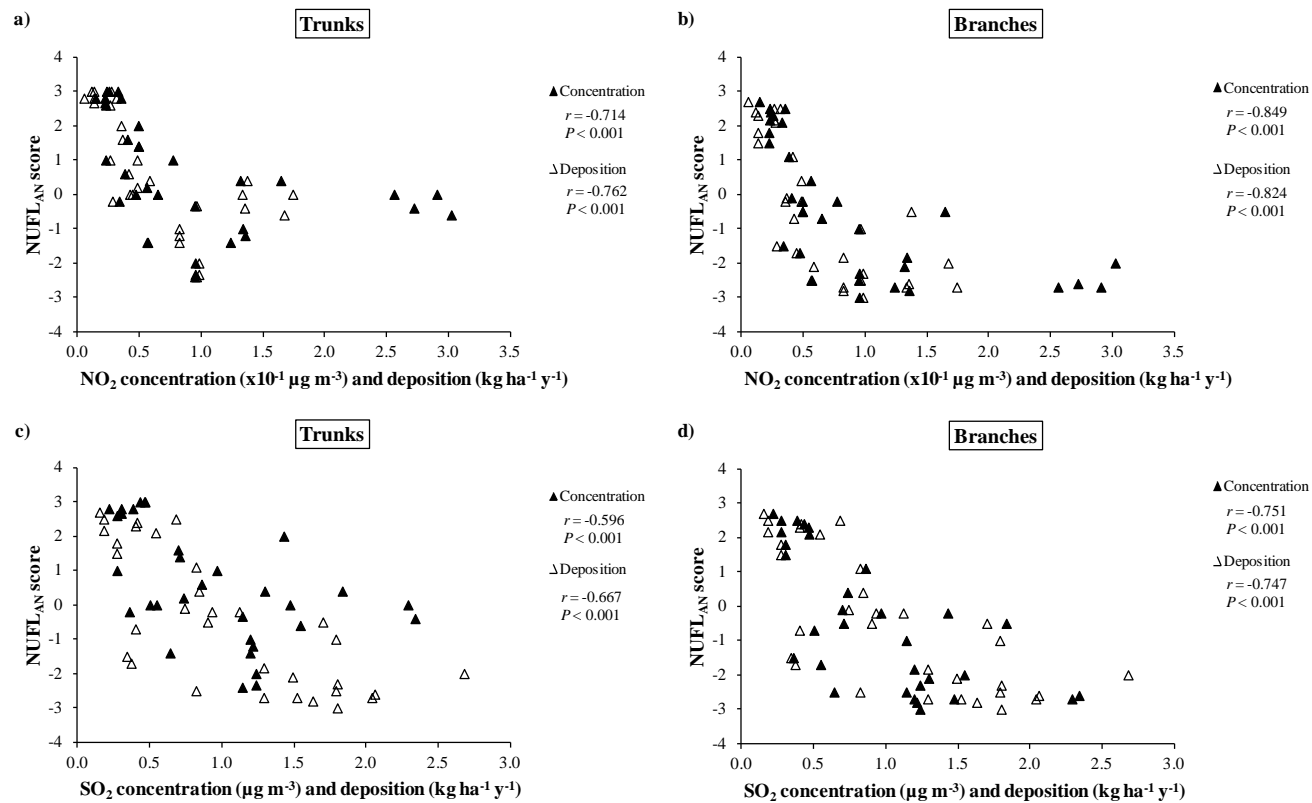
### 5.3.2.2 Relationships between NUFL<sub>AN</sub> Scores and Atmospheric Chemistry

As expected, a strong relationship existed between [NH<sub>3</sub>] values and NUFL<sub>AN</sub> scores for both trunks and branches (Figures 5.4a and 5.4b). High correlations on both substrata were also found between NUFL<sub>AN</sub> scores and [NH<sub>4</sub><sup>+</sup>] (Figures 5.4 c and 5.4d). The strong relationship between [NH<sub>4</sub><sup>+</sup>] and NUFL<sub>AN</sub> scores could be explained by the correlation (PPMCC;  $n = 28$ ;  $r = 0.650$ ;  $P < 0.001$ ) observed between [NH<sub>3</sub>] and [NH<sub>4</sub><sup>+</sup>] values (Section 3.3.5). The key variable of these two was accepted as being [NH<sub>3</sub>], given that the indicator species used in this analysis were derived from an Indicator Species Analysis (ISA) based upon [NH<sub>3</sub>].

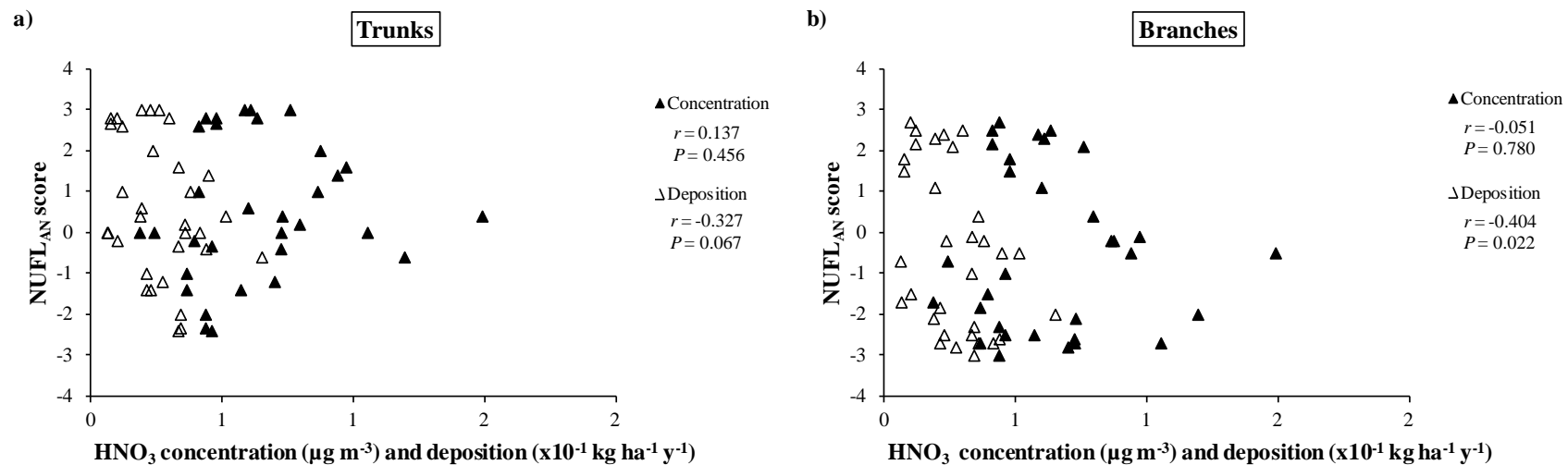
The concentration and deposition values of both NO<sub>2</sub> and SO<sub>2</sub> were seen to correlate with NUFL<sub>AN</sub> scores (Figure 5.5). However, as with the previous case, only one of these pollutants was accepted as being legitimately associated with NUFL<sub>AN</sub> scores. NO<sub>2</sub> was determined as the main factor based upon three key points. First, the relationships with NUFL<sub>AN</sub> scores were always strongest for NO<sub>2</sub> than SO<sub>2</sub>. Second, all the score relationships with NO<sub>2</sub> and SO<sub>2</sub> were negative. This trend was more typical of a lichen response to N-enrichment, or raised bark pH, rather than S-based pollution or acidification. Finally, earlier analysis (see Section 3.3.5) identified a strong positive correlation between NO<sub>2</sub> and SO<sub>2</sub> values at survey sites (PPMCC;  $n = 28$ ;  $r = 0.919$ ;  $P < 0.001$ ). Consequently, the relationships observed between SO<sub>2</sub> concentration in air and deposition values and NUFL<sub>AN</sub> scores were deemed to be a result of co-correlation of the pollutant with NO<sub>2</sub>.



**Figure 5.4** Relationships between NUFL<sub>AN</sub> scores and the concentration and deposition of a,b) NH<sub>3</sub> and c,d) NH<sub>4</sub><sup>+</sup> on trunks and branches. Pearson's Product Moment Correlation Co-efficient values are given. Concentration values were square root transformed and NH<sub>4</sub><sup>+</sup> deposition values Box-Cox transformed prior to analysis.



**Figure 5.5** Relationships between NUFL<sub>AN</sub> scores and the concentration and deposition of (a,b) NO<sub>2</sub> and (c,d) SO<sub>2</sub> on trunks and branches. Pearson's Product Moment Correlation Co-efficient values are given. Concentration values were square root transformed for NO<sub>2</sub> and all SO<sub>2</sub> values Box-Cox transformed prior to analysis.

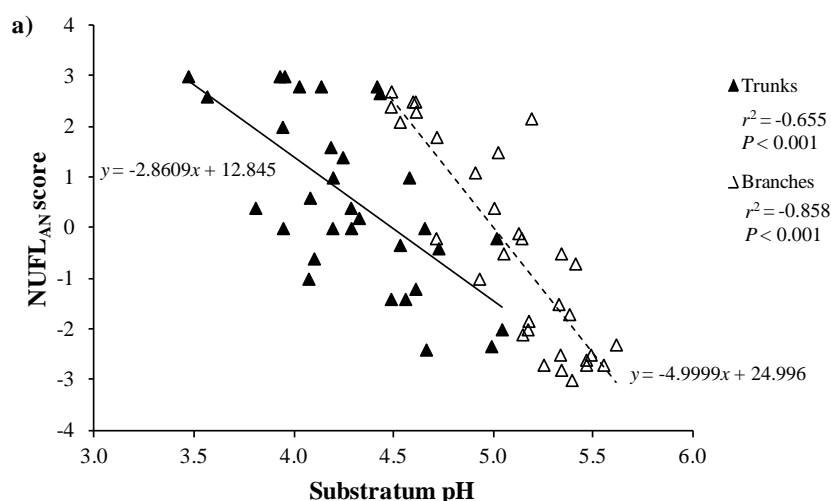


**Figure 5.6** Relationships between NUFL<sub>AN</sub> scores and the concentration and deposition of HNO<sub>3</sub> on (a) trunks and (b) branches. Pearson's Product Moment Correlation Co-efficient values are given. Concentration values were square root transformed prior to analysis.

NUFL<sub>AN</sub> scores for trunks and branches showed strong correlations with deposition of NO<sub>2</sub> and SO<sub>2</sub> (Figure 5.5), but not with deposition of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> (Figure 5.4). Comparison of the strength of relationships between NO<sub>2</sub> in concentration and deposition against NUFL<sub>AN</sub> scores showed a slightly stronger correlation on branches compared to trunks (Figures 5.5a and 5.5b). No significant relationships were observed between NUFL<sub>AN</sub> scores and HNO<sub>3</sub> values in the form of either concentration or deposition (Figure 5.6).

### **5.3.2.3 Relationships between NUFL<sub>AN</sub> Scores and Bark pH**

The NUFL<sub>AN</sub> score was strongly negatively related to bark pH (Figure 5.7), suggesting that this variable should be considered a confounding factor together with RD > 1 mm (Section 5.3.1) and [NO<sub>2</sub>] (Section 5.3.2). Linear regression lines fitted to the data showed that the gradient of the regression line was much steeper on the branches, implying a potentially greater pH sensitivity of lichens growing on this substratum. The point at which scores became dominated by nitrophilic species along the lines of regression (i.e. NUFL<sub>AN</sub> = 0) for trunks and branches appear to differ, with the change of dominant indicator species type occurring at a lower pH for trunks (pH 4.5) than branches (pH 5.0). However, this should not be accepted as fact due to the different techniques used to measure the substratum pH of trunks and branches (see Section 2.12 for details).



**Figure 5.7** Relationships between NUFL<sub>AN</sub> scores and bark pH on trunks and branches. Regression lines (solid line, trunks; dashed line, branches) indicated a strong negative relationship on both substrata.

**Table 5.1** Correlation analysis between bark pH of trunks and branches on *Betula* and *Quercus* trees and mean monthly [NH<sub>3</sub>], [NO<sub>2</sub>] and [SO<sub>2</sub>] in air. PPMCC analysis. Significant relationships at the  $P = 0.05$  are shown in bold type.

Phorophyte	Bark pH	[NH <sub>3</sub> ] <sup>*</sup>		[NO <sub>2</sub> ] <sup>†</sup>		[SO <sub>2</sub> ] <sup>*</sup>	
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
All	Trunk	<b>0.620</b>	<b>&lt; 0.001</b>	0.289	0.109	0.229	0.207
	Branch	<b>0.726</b>	<b>&lt; 0.001</b>	<b>0.628</b>	<b>&lt; 0.001</b>	<b>0.547</b>	<b>0.001</b>
<i>Betula</i>	Trunk	<b>0.626</b>	<b>0.039</b>	0.528	0.095	0.432	0.185
	Branch	<b>0.835</b>	<b>0.001</b>	<b>0.681</b>	<b>0.021</b>	0.589	0.056
<i>Quercus</i>	Trunk	<b>0.433</b>	<b>0.050</b>	0.085	0.713	0.028	0.905
	Branch	<b>0.562</b>	<b>0.008</b>	<b>0.435</b>	<b>0.049</b>	0.299	0.188

\* Based on log<sub>10</sub> transformed data

† Based on Box-Cox transformed data

Bark pH analysis of trunks and branches for *Betula* and *Quercus* trees, both combined and separated by genera, against atmospheric chemistry variables identified several significant positive relationships (Table 5.1). Most notable was the relationship observed between substratum pH and [NH<sub>3</sub>]. Significant



positive relationships also existed between branch pH values and  $[\text{NO}_2]$ , but little significance was seen for  $[\text{SO}_2]$  levels.

The first two variables entered into the model were  $[\text{NH}_3]$  and  $[\text{NO}_2]$ , which possessed the highest  $r^2$  values for trunks and branches respectively. Both variables also had a negative relationship with the  $\text{NUFL}_{\text{AN}}$  (Figures 5.4 and 5.5), which meant that their values in the model were additive to each other. The second stage of the model construction involved implementing bark pH of the trunks and branches to the relevant equations. The pH values were also added to the established  $a[\text{NH}_3] + b[\text{NO}_2]$  optima established given the negative correlation between pH and  $\text{NUFL}_{\text{AN}}$  scores (Figure 5.7). The final procedure of the modelling involved introducing  $\text{RD} > 1 \text{ mm}$  into the equation. As a variable positively correlated with  $\text{NUFL}_{\text{AN}}$  scores (Figure 5.2),  $\text{RD} > 1 \text{ mm}$  values were subtracted from the other parts of the equation model.

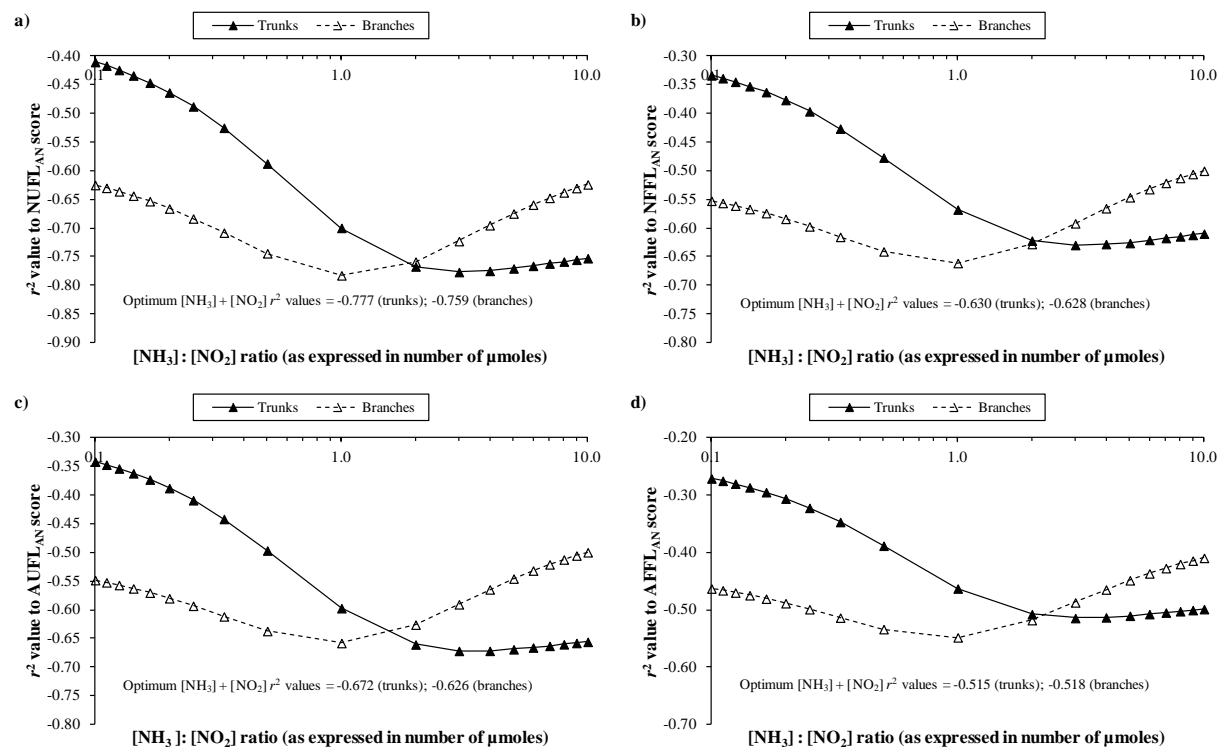
### **5.3.3 Formulation of an Optimum Model for the $\text{NUFL}_{\text{AN}}$ Scoring System**

The sum of the two [N] compounds provided a higher  $r^2$  value than either  $[\text{NH}_3]$  or  $[\text{NO}_2]$  did individually (Figure 5.8), implying an additive effect of the two N forms. The optimum  $r^2$  for the  $[\text{NH}_3] + [\text{NO}_2]$  model, occurred when  $[\text{NH}_3]:[\text{NO}_2]$  was weighted in a  $3:1 \mu\text{mol m}^{-3}$  ratio for trunks and a  $1:1 \mu\text{mol m}^{-3}$  ratio for branches. Therefore, in both these instances  $[\text{NH}_3]$  was observed as being the more important variable, however,  $[\text{NO}_2]$  became a more influential factor on the branches compared to the trunks. The ratios of  $a[\text{NH}_3] + b[\text{NO}_2]$  were the same for all four  $\text{FL}_{\text{AN}}$  scoring methods, but the

more heavily SR-weighted approaches possessed the lowest  $r^2$  values (Fig 5.8). Trunk and branch optima ranged from  $r^2 = -0.515$  to  $r^2 = -0.777$  and  $r^2 = -0.548$  to  $r^2 = -0.783$  respectively, with  $r^2$  values for the scoring methods decreasing in the order  $\text{NUFL}_{\text{AN}} > \text{AUFL}_{\text{AN}} > \text{NFFL}_{\text{AN}} > \text{AFFL}_{\text{AN}}$  in both instances. The two SR-weighted scoring systems calculated through the multiplication of number of species at each site (i.e. the  $\text{NFFL}_{\text{AN}}$  and  $\text{AFFL}_{\text{AN}}$ ) possessed the weakest relationships (Figure 5.8). This lends further support to the idea that weighting for SR reduces the effectiveness of a  $\text{FL}_{\text{AN}}$ -based technique, and that a simple un-weighted approach, such as the  $\text{NUFL}_{\text{AN}}$  is potentially more reliable.

As with the initial stage of the model, a further increment in  $r^2$  values was observed with the addition of bark pH as the third variable (Figure 5.9). The relationship between the  $(a[\text{NH}_3] + [\text{NO}_2]) + \text{pH}$  model and  $\text{NUFL}_{\text{AN}}$  scores increased to  $r^2 = -0.813$  for trunks, and  $r^2 = -0.877$  for branches, an increase of 0.036 and 0.094 respectively compared to the initial model. Concentrations of N, as represented by  $[\text{NH}_3] + [\text{NO}_2]$ , had a greater influence than pH for the trunks with an  $(a[\text{NH}_3] + [\text{NO}_2]) : \text{pH}$  ratio of 2:1. However, this fell to a ratio of 1.5:1 on the branches. Addition of  $\text{RD} > 1 \text{ mm}$  to the model only resulted in very small increases to the established model, with correlation values against  $\text{NUFL}_{\text{AN}}$  scores rising from  $r^2 = -0.813$  to  $r^2 = -0.820$  for trunks, and from  $r^2 = -0.877$  to  $r^2 = -0.879$  for branches. The  $a(b[\text{NH}_3] + [\text{NO}_2]) + \text{pH}$  to  $\text{RD} > 1 \text{ mm}$  ratio was also observed to be very high, with ratios of 175:1 and 750:1 being observed for the trunks and branches respectively. Consequently,

the equations used for both trunks (Eq. 5.2) and branches (Eq. 5.3) excluded climate.



**Figure 5.8** Modelling of weighting between  $[\text{NH}_3]$  and  $[\text{NO}_2]$  ratios against (a) NUFL<sub>AN</sub>, (b) NFFL<sub>AN</sub>, (c) AUFL<sub>AN</sub> and (d) AFFL<sub>AN</sub> scores on trunks and branches at survey sites. Linear regression analysis showed similar patterns for all scoring techniques. The strongest relationship was observed for the model applying a three-fold and one to one  $[\text{NH}_3] : [\text{NO}_2]$  ratio (as calculated in number of micro moles) to trunks and branches respectively.

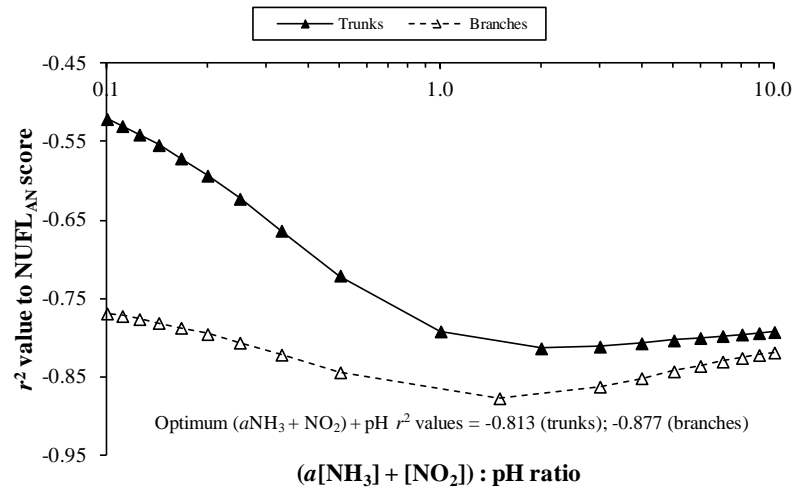
The modelled  $\text{NUFL}_{\text{AN}}$  values, calculated from the formulated regression equations (Eq. 5.2 and 5.3) using the  $[\text{NH}_3]$ ,  $[\text{NO}_2]$  and pH values obtained from each site, showed strong relationships with the measured  $\text{NUFL}_{\text{AN}}$  values (Figure 5.10). This procedure was repeated for a universal model that was derived from the mean  $[\text{NH}_3]:[\text{NO}_2]:\text{pH}$  ratios of the trunk and branch equations (Eq. 5.4). Linear regression analysis of the universal model showed that it also possessed strong relationships to measured  $\text{NUFL}_{\text{AN}}$  values, indicating that it could be used for both substrata (Figure 5.10).

$$\text{Trunk model}^* = -\{2(3 \cdot [\text{NH}_3]) + 1 \cdot [\text{NO}_2]) + \text{pH}\} \quad [\text{Eq. 5.2}]$$

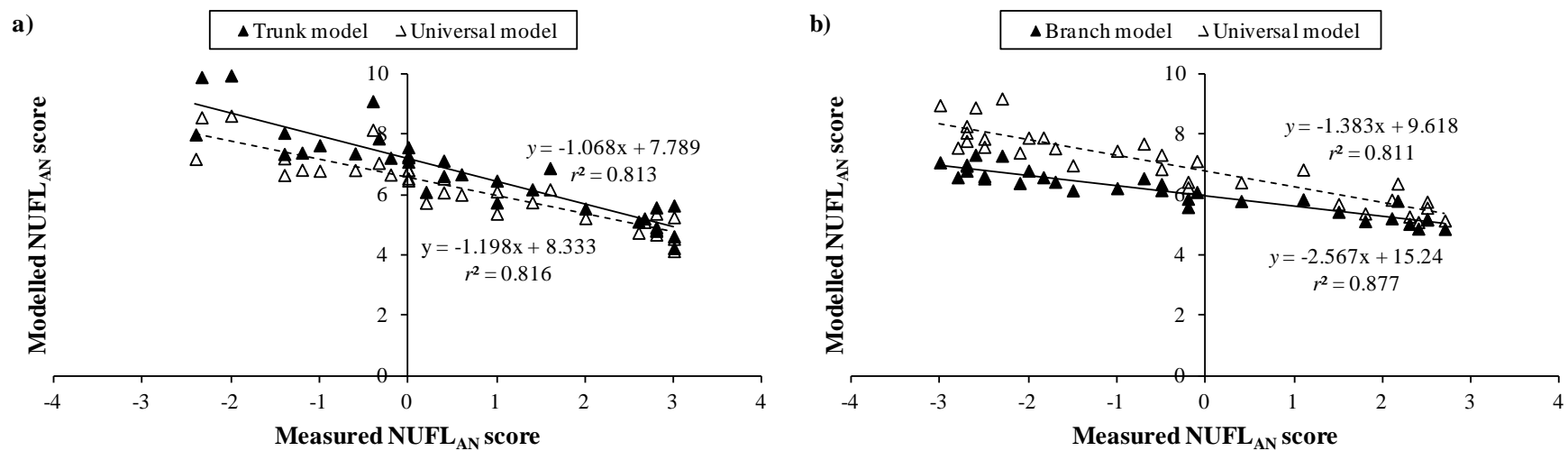
$$\text{Branch model}^* = -\{1.5(1 \cdot [\text{NH}_3]) + 1 \cdot [\text{NO}_2]) + \text{pH}\} \quad [\text{Eq. 5.3}]$$

$$\text{Universal model}^* = -(5 \cdot [\text{NH}_3] + 2 \cdot [\text{NO}_2]) + \text{pH} \quad [\text{Eq. 5.4}]$$

\*  $[\text{NH}_3]$  and  $[\text{NO}_2]$  represented by the number of  $\mu\text{mol m}^{-3}$  of each compound.



**Figure 5.9** Modelling of the optimum  $(a[\text{NH}_3] + [\text{NO}_2]) + \text{pH}$  value, where  $a = 3$  for trunks, and  $a = 1$  for branches. A regression analysis was undertaken between  $\text{NUFL}_{\text{AN}}$  scores and varying ratios of  $(a[\text{NH}_3] + [\text{NO}_2]) : \text{pH}$  for survey sites to find the proportions of all three variables that provided the optimum  $r^2$  value. Optimums were observed at the 2:1 and 1.5:1 ratios respectively for trunks and branches, in favour of  $(a[\text{NH}_3] + [\text{NO}_2])$  over pH.



**Figure 5.10** Relationships between modelled and measured  $\text{NUFL}_{\text{AN}}$  scores using the individual optimum and universal equations for trunks (a) and branches (b) across surveyed sites. The universal model ( $\text{NUFL}_{\text{AN}} = -(4 \cdot [\text{NH}_3] + 2 [\text{NO}_2] + \text{pH})$ ) was derived from the best fitting compromise equation between the individual optima for trunks ( $\text{NUFL}_{\text{AN}} = -(2(3 \cdot [\text{NH}_3] + [\text{NO}_2]) + \text{pH})$ ) and branches ( $\text{NUFL}_{\text{AN}} = -(1.5(\cdot [\text{NH}_3] + \cdot [\text{NO}_2]) + \text{pH})$ ).

#### 5.3.4 Influence of Phorophyte Type on NUFL<sub>AN</sub> Scores

*Betula* trees produced stronger correlations with NUFL<sub>AN</sub> scores than *Quercus* for both trunks and branches (Figure 5.11), and also produced more positive NUFL<sub>AN</sub> scores than *Quercus* at four of the five sites where both tree genera were surveyed (Figure 5.12). The only exception to this was Hinderclay Fen.

The branches of *Betula* trees across the 28 survey sites had a mean pH of 4.82, which was significantly more acidic than the 5.23 mean pH for *Quercus* branches (Table 5.2). No significant difference existed on the trunks between the two tree genera, where mean pH values were 4.25 and 4.32 for *Betula* and *Quercus* respectively. A more accurate assessment using the limited number of five sites where pH was measured for both genera also produced no significant results (Table 5.3); however a pattern emerged with the mean bark pH on *Betula* (pH 4.26 trunks and pH 4.96 branches) still being consistently more acidic than that of *Quercus* (pH 4.56 trunks and pH 5.34 branches).

In addition to the above analysis, a multiple linear regression was undertaken between trunk bark pH and [NH<sub>3</sub>] and the potentially confounding effect of tree age (represented by Bt and gbh). The results of this indicated that tree age was not significantly related to pH across all survey sites (Table 5.4) in all cases except for *Quercus* gbh, where substratum pH became more acidic with increasing girth.

**Table 5.2** Comparison of surface bark pH on the trunks (a) and branches (b) of *Betula* and *Quercus* trees at the 28 survey sites. A significant difference (one-way ANOVA;  $P < 0.001$ ) in mean pH was observed on branches between the two tree genera, but not on the trunks.

a)	Source	DF	SS	MS	F	P
	Trunk pH	1	0.037	0.037	0.24	0.625
	Error	30	4.563	0.152		
	Total	31	4.6			
	S = 0.3900 R-Sq = 0.81% R-Sq(adj) = 0.00%					

b)	Source	DF	SS	MS	F	P
	Branch pH	1	1.2304	1.2304	15.54	< 0.001
	Error	30	2.3754	0.0792		
	Total	31	3.6058			
	S = 0.2814 R-Sq = 34.12% R-Sq(adj) = 31.93%					

**Table 5.3** Comparison of surface bark pH on the trunks (a) and branches (b) of *Betula* and *Quercus* trees at five sites where measurements were taken for both tree genera. No significant difference (one-way ANOVA;  $P < 0.05$ ) in mean pH was observed on either substratum.

a)	Source	DF	SS	MS	F	P
	Trunk pH	1	0.232	0.232	1.21	0.304
	Error	8	1.539	0.192		
	Total	9	1.771			
	S = 0.4386 R-Sq = 13.10% R-Sq(adj) = 2.24%					

b)	Source	DF	SS	MS	F	P
	Branch pH	1	0.3629	0.3629	4.33	0.071
	Error	8	0.6709	0.0839		
	Total	9	1.0338			
	S = 0.2896 R-Sq = 35.10% R-Sq(adj) = 26.99%					

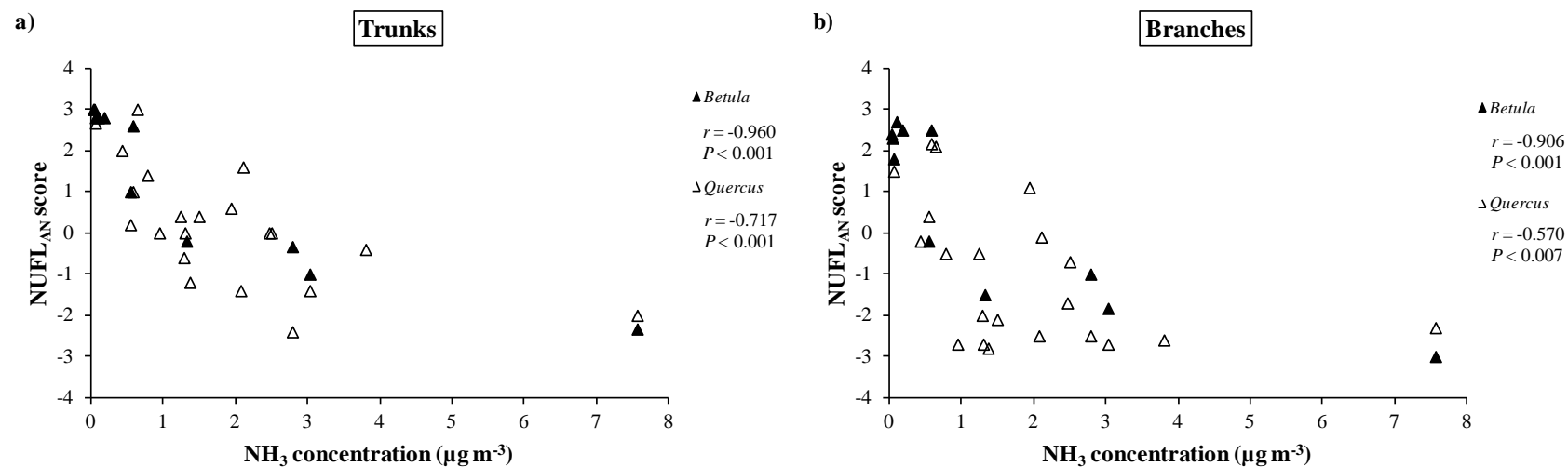
**Table 5.4** Multiple linear regressions of the relationship between substratum pH and the three variables mean monthly annual  $[\text{NH}_3]$  values, bark rugosity and tree girth on a) *Quercus*, and b) *Betula* tree boles at the 28 survey sites.

a)	Predictor	Coef	SE Coef	T	P
	Substratum pH	4.3920	0.1116	39.35	< 0.001
	$[\text{NH}_3]$	0.1140	0.02346	4.86	< 0.001
	Bt	-0.0325	0.1209	-0.27	0.788
	gbh	-0.1131	0.04508	-2.51	0.014
	S = 0.342491 R-Sq = 30.6% R-Sq (adj) = 28.4%				

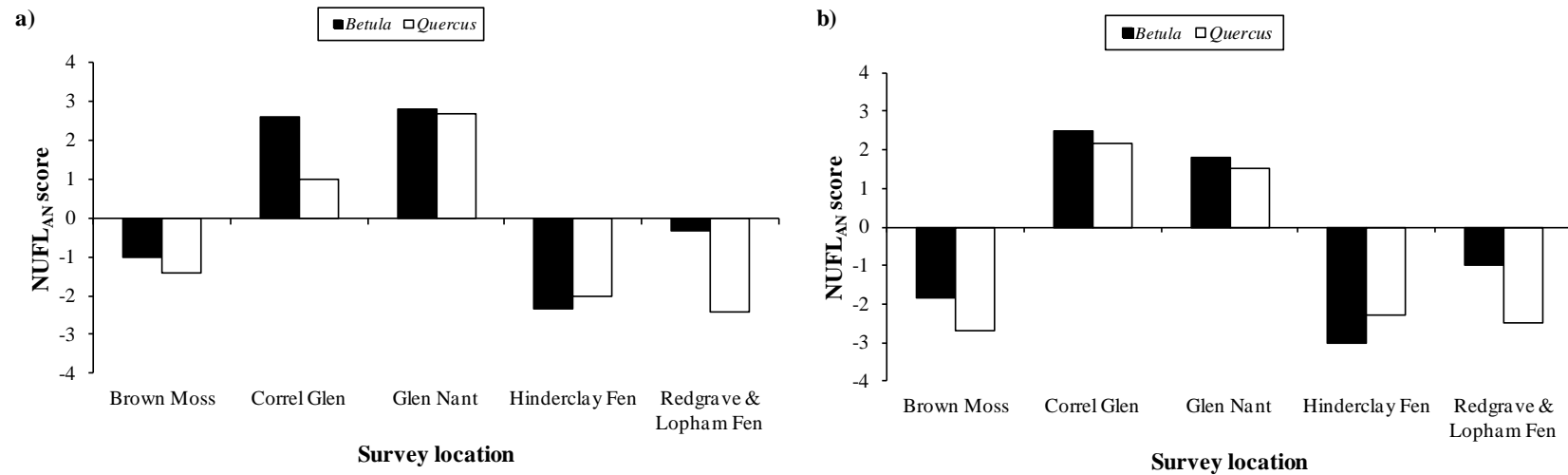
  

b)	Predictor	Coef	SE Coef	T	P
	Substratum pH	4.2991	0.2549	16.86	< 0.001
	$[\text{NH}_3]$	0.0969	0.03431	2.82	< 0.001
	Bt	0.2225	0.1471	1.51	0.137
	gbh	-0.1737	0.1782	-0.97	0.334
	S = 0.347488 R-Sq = 54.8% R-Sq (adj) = 51.0%				





**Figure 5.11** Relationships between atmospheric  $\text{NH}_3$  concentrations and NUFL<sub>AN</sub> scores on (a) trunks and (b) branches of *Betula* and *Quercus* trees. Pearson's Product Moment Correlation Co-efficient values are given.



**Figure 5.12** Intersite differences in NUFL<sub>AN</sub> scores between *Betula* and *Quercus* on (a) trunks and (b) branches at five regional-scale survey sites. Scores for *Betula* were consistently more positive than those of *Quercus* trees, implying a fundamental difference between the two phorophytes.

## 5.4 Discussion

### 5.4.1 Assessment of FL<sub>AN</sub> Scoring System

All four FL<sub>AN</sub> scores were strongly correlated with [NH<sub>3</sub>] values, with the NUFL<sub>AN</sub> showing the strongest relationship (Figure 5.1). The NUFL<sub>AN</sub> is the only method not weighted for SR, which implies that weighting for SR causes skewing of scores. This is supported by the observation that correlations were weakest for the AFFL<sub>AN</sub>, where SR weighting was both additive and factorial. The influence of SR was less for both the NFFL<sub>AN</sub> and the AUFL<sub>AN</sub> compared to the AFFL<sub>AN</sub> as SR had either a solely factorial or additive effect on the two scores respectively.

It is possible that the results for the three SR weighted approaches may have encountered bias because of inconsistencies in the indicator species shortlist (Table 4.10). For example, the grouping of *Physcia adscendens* and *P. tenella*, and the aggregation of *Xanthoria candelaria* s.s. and *X. ucrainica* into *X. candelaria* s.l. would have no effect on the purely frequency-based NUFL<sub>AN</sub>, but bias the other three approaches. It is conceivable that separating these species would improve the fit of the three SR-weighted FL<sub>AN</sub> scoring systems. However, doing so would risk corruption of the weighted scoring systems through misidentification.

Further to this, the SR weighted approaches naturally skew the scale to give disproportionately higher scores to sites with higher SR (in relation to the indicator species list). This means that sites with a lower SR as a result of past perturbations resulting from either land management or non-N based

environmental pollution, such as  $[\text{SO}_2]$  (Hawksworth & Rose, 1970; McCune, 1988; Nash III, 1973), could be unfavourably scored due to limited or randomised re-colonisation rates of species of expected communities (Hawksworth & McManus, 1989), possibly as a result of natural time lags in species re-colonisation or disruption to facilitative processes. This effect should be minimised on the purely frequency-based  $\text{NUFL}_{\text{AN}}$ , compared to the other three SR dependent approaches, but was possibly still observed, as is described in the following case.

$\text{NUFL}_{\text{AN}}$  scores for trunks and branches differed at each site, with trunk scores typically being more positive. Two explanations for this disparity are the potentially more acidic bark substratum of trunks and their considerably greater longevity to branches. The latter reason suggests that the trunk and branch scores can provide complementary information on the temporal condition of a site, but this was not always shown to be true. An example of this was shown at Bretton Lakes. Cattle numbers at the surveyed site were reduced from over 40 to four in recent years (A. Mackenzie, Pers. comm., 2009). Thus, mean monthly  $[\text{NH}_3]$  value of  $0.94 \mu\text{g m}^{-3}$  measured between 2009 and 2010 are likely to be much lower than previous concentrations for the site. Therefore, lower  $\text{NUFL}_{\text{AN}}$  scores would theoretically be expected on the trunks than branches since they should reflect the past site conditions of higher  $[\text{NH}_3]$  values. It is conceivable that this prediction might be compromised by nitrophobic indicator species persistency on trunks resulting from factors such as the buffering capacity of a lower substratum pH (all tree trunks at the site possessed a  $\text{pH} < 4.5$ ), or the time lag for all established individuals to be lost

at the site by means of an extinction debt (Berglund *et al.*, 2005; Metzger *et al.*, 2009) of N-sensitive species. However, observations should theoretically be made whereby a larger proportion of nitrophilic species should be present on the trunks, or at least the older sections of branches, compared to the 1-5 and 6-10 year branch lengths. But this was not found to be the case.

Observations of the  $NUFL_{AN}$  at Bretton Lakes showed that scores were more positive for trunks ( $NUFL_{AN} = 0.0$ ) than branches ( $NUFL_{AN} = -2.7$ ). However, this was due to nitrophilic indicator dominance on branches rather than nitrophobic indicator persistency on trunks, as a total absence of both indicator species was observed on the trunks. Also, the dominance of nitrophilic indicators on the branches was particularly noticeable in the most recent 10 years growth. Both nitrophilic (*Lecidella elaeochroma*, *Phycia tenella*, *Xanthoria candelaria* s.l., *X. parietina* and *X. polycarpa*) and nitrophobic (*Evernia prunastri*) indicators were recorded on the 11+ year sections of the branches, whereas only nitrophiles (*Phycia tenella*, *Xanthoria candelaria* s.l., *X. parietina* and *X. polycarpa*) were recorded on the 1-5 and 6-10 age classes.

Two key conclusions can be drawn from this. First, the absence of nitrophobes on the trunks could be the result of either past disturbance through  $[NH_3]$ ,  $[SO_2]$  or a combination of both, whilst nitrophile absence was likely due to either past  $[SO_2]$ , high bark acidity, or a combination of these two factors. Second, nitrophobe absence on the 1-5 and 6-10 years, combined with the widespread presence of nitrophilic indicators on these branch sections implies that the substratum pH is naturally amenable for nitrophilic species

colonisation. It also provides evidence of a time lag in species colonisation and relative to changes in atmospheric [N] that have not hitherto been factored into a lichen bio-monitoring scheme. As a result, despite the greater robustness of the  $\text{NUFL}_{\text{AN}}$  compared to the other frequency-based techniques, the results can still be influenced by factors other than current [N] levels. Further to this, it cannot be dismissed that nitrophobe absence on the 1-5 and 6-10 age classes of branches could be an aberration resulting from limited sampling (see Section 4.5).

One final important point regarding the assessment of the scoring system is that a very basic approach was used, wherein indicator frequencies were tallied based upon presence or absence on three aspects of each tree. This was done primarily to balance the scores for trunks with those of branches, but also provided insight into the feasibility of a very basic sampling approach. Although the findings of this study imply this approach is capable of producing a robust relationship between indicator species and  $[\text{NH}_3]$ , the lack of resolution in the survey means that the presence of a single thallus of one indicator class (e.g. nitrophobe) can counter the presence of many thalli of numerous different species of the opposing indicator class (e.g. nitrophile). This is a particular problem when using the  $\text{NUFL}_{\text{AN}}$  scoring system as it is not weighted for SR. However, if the ladder transect approach is adopted when surveying trunks (i.e. recording species in five separate 10 x 10 cm quadrats, instead of one general recording, per aspect) this will prevent excessive weighting for species rarely occurring at a site.

#### 5.4.2 Identification of Confounding Environmental Variables

Correlation analysis of the NUFL<sub>AN</sub> scoring system (as established from a shortened list of NH<sub>3</sub> indicator species) and [NH<sub>3</sub>] showed that the NUFL<sub>AN</sub> provided a reliable assessment tool for estimating [NH<sub>3</sub>] at a site. In addition to [NH<sub>3</sub>], scores were also correlated with seven other variables: [NH<sub>4</sub><sup>+</sup>], [SO<sub>2</sub>], [NO<sub>2</sub>], bark pH, climate and NO<sub>2</sub> and SO<sub>2</sub> deposition (Figures 5.2, 5.4, 5.5 and 5.7). In an attempt to simplify the model, [NH<sub>4</sub><sup>+</sup>], [SO<sub>2</sub>] and NO<sub>2</sub> and SO<sub>2</sub> deposition were excluded under the supposition that their relationships were due to co-correlation with the other variables. A good example of this was [SO<sub>2</sub>], which co-varied with [NO<sub>2</sub>] and showed the same negative correlation with NUFL<sub>AN</sub> scores, even though the inverse relationship between [SO<sub>2</sub>] and NUFL<sub>AN</sub> would be expected. Relationships between NUFL<sub>AN</sub> scores and deposition of NO<sub>2</sub> and SO<sub>2</sub> were also accepted as being a result of co-correlation with the air concentrations of the respective chemicals. However, it should be noted that this may provide evidence of the importance of stemflow as a vector of nutrients and air pollution transfer to lichen thalli on trunks where the effect of deposition is significant, as was displayed in work on epiphytic bryophytes by Leith *et al.* (2008).

The three variables factored in with [NH<sub>3</sub>] when formulating the model were: climate (as defined by RD > 1 mm), [NO<sub>2</sub>] and bark pH. [NO<sub>2</sub>] and climate were included based on previous assessments with [NH<sub>3</sub>] (Sections 3.3.5 and 5.5.2 respectively), and the only other notable inter-variable correlation observed was between [NO<sub>2</sub>] and branch pH (Table 5.1). Correlations between substratum pH and the [NO<sub>2</sub>] co-variant [SO<sub>2</sub>] were also observed (Table 5.1),

but contrary to expectations, the relationship was positive, indicating that [SO<sub>2</sub>] was not directly influencing pH and that any relationship was likely due to [NO<sub>2</sub>] (see Section 3.3.5 for details).

Bark pH on the trunks and branches of both *Betula* and *Quercus* showed strong positive correlations with [NH<sub>3</sub>] (Table 5.1), and this finding is supported by previous studies (de Bakker, 1989; Frati *et al.*, 2007; van Herk, 2001). It has been suggested by van Herk, (1999) that the presence of N-tolerant species is determined by increases in substratum pH driven by [NH<sub>3</sub>]. In contrast, Wolseley *et al.* (2005<sup>a</sup>) suggest that N has an effect on lichens independent of that mediated by bark pH, and that a stronger relationship is shown when both [NH<sub>3</sub>] and bark pH are considered together. Another observation by Wolseley *et al.* (2005<sup>a</sup>) was that differences between trunk and branch indicator scores could be explained by differences in pH between the two substrata. However, this statement is potentially misleading because, as in the case of the current study (see Section 2.12 for details), two different methods were used to measure the pH of trunks and twigs (Wolseley *et al.*, 2005<sup>b</sup>). It would therefore be erroneous to take this explanation as a definitive conclusion based on these results. Likewise in this study, the possibility that differences in pH for the two substrata where NUFL<sub>AN</sub> are the same (Figure 5.7) is an artefact of the different methodological approaches used in pH measurements must be considered.



### 5.4.3 Formulation of an Optimum Model for the NUFL<sub>AN</sub> Scoring System

The step-by-step construction of the model showed a continued increase in the amount of variation explained with the addition of each variable. This supports the notion that each variable was influential for indicator species presence, and supports the statement by Wolseley *et al.* (2005<sup>a</sup>) that [NH<sub>3</sub>] and bark pH independently influence lichen community composition. The corresponding independent relationship between [NO<sub>2</sub>] and NUFL<sub>AN</sub> scores provides evidence that the shortlisted indicator species selected may be useful as [NO<sub>2</sub>] indicator species in conjunction with their use as [NH<sub>3</sub>] indicators. Therefore they might act as a baseline for future work investigating the transferral of this method to more urban environments, where N sources are predominantly in the oxidised form.

Addition of climate as the fourth factor did little to improve the relationship. Hence, this was dropped on the premise that most variation could be explained by the three variables [NH<sub>3</sub>], [NO<sub>2</sub>] and bark pH alone. Consequently, this led to the formation of two equations for model optima based upon the three variables of [NH<sub>3</sub>], [NO<sub>2</sub>] and pH for trunks (Eq. 5.2) and branches (Eq. 5.3). The amalgamation of these two models into a universal equation (Eq. 5.4) also correlated strongly with the measured NUFL<sub>AN</sub> values for both trunks and branches (Figure 5.10). This meant that, once the NUFL<sub>AN</sub> score was determined and both bark pH and [NO<sub>2</sub>] values were obtained, a universal model for use in the bio-monitoring scheme could be applied to either substratum to estimate levels of the spatially variable [NH<sub>3</sub>].

#### 5.4.4 Influence of Phorophyte Type on the NUFL<sub>AN</sub>

A considerably stronger relationship with [NH<sub>3</sub>] was observed between *Betula* NUFL<sub>AN</sub> scores than those of *Quercus* (Figure 5.11). Two distinct differences were identified between the phorophytes surveyed in this study: NUFL<sub>AN</sub> scores were typically more positive (Figure 5.12), and bark pH more acidic, on *Betula* compared to *Quercus*. Given the established relationship between bark pH and NUFL<sub>AN</sub> scores it seems likely that the recorded phorophyte differences were the result of bark pH differences between the tree genera, as the typically more acidic *Betula* trees possessed a greater buffering capacity against deposition of the alkaline gas [NH<sub>3</sub>]. Differences in bark pH occur as a result of specific variation between the tree genera (Barkman, 1958<sup>a</sup>), but environmental influences, such as leaching may also play a role. The weaker relationship between NUFL<sub>AN</sub> scores and *Quercus* trees may result from greater variability in pH resulting from a more complex relationship with the environment.

The relationship shown between bark pH and tree girth (Table 5.4) implies that acidification occurs over time due to leaching, and this may be more significant for *Quercus* trees given their greater age and general longevity (Jones, 1959). Consequently, the *Betula* results might prove more reliable as the confounding influence of increasing bark acidity due to leaching over time is not as significant a factor in offsetting the [NH<sub>3</sub>] influenced basification of bark pH. Furthermore, the historical effect of bark acidification and lichen loss through previous [SO<sub>2</sub>] pollution is likely to have a greater effect on *Quercus* than *Betula*. This is because, under the assumption that the *Quercus* trees were

older, they would have a greater exposure time to past [SO<sub>2</sub>] effects. Should the hypothesis be upheld that temporal exposure to the environment weakens the relationship of *Quercus* trees to the NUFL<sub>AN</sub> score, this could be offset by surveying *Quercus* trees within a predetermined age range.

## **Chapter 6. Influence of N Deposition on Cryptogamic Species: Results from a Field Manipulation Experiment**

### **6.1 Introduction**

Research investigating N effects on epiphytes in this thesis in terms of production of the bio-monitoring tool has focussed primarily on  $\text{NH}_3$  and  $\text{NO}_2$ . Both of these gases deposit over short distances from low level point sources by dry deposition (NEG-TAP, 2001). Other forms of atmospheric inorganic N that have been paid less attention include wet deposited  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . It has been remarked by van Herk *et al.* (2003<sup>a,b</sup>) that the potential influence of these two ions upon epiphytic lichen communities requires some attention. A study of the effects of wet N deposition enrichment in arctic heathland by Fremstad *et al.* (2005) showed that lichens such as *Alectoria nigricans*, *Cetraria ericetorum* and *Cladonia mitis* declined when exposed to an increase in  $\text{NH}_4\text{NO}_3$ . In such terricolous communities it is uncertain whether effects on lichens are due to direct toxic effects or competitive exclusion by co-occurring vascular plants. The effects of N enrichment on epiphytes are less likely to be confounded by vascular plant responses.

Since  $\text{NH}_4^+$  is a cation and  $\text{NO}_3^-$  is an anion, investigating the effect of these two ionic N forms on substratum pH and lichen community composition might provide an insight into the importance of N deposition to cryptogams, both directly and through perturbation to substratum pH. The effect of  $\text{NH}_4^+$  on cryptogamic communities would be of particular interest given that it is the

conjugate acid of  $\text{NH}_3$ . By comparing the community composition of epiphytes exposed to  $\text{NH}_4^+$  only against those exposed to  $\text{NH}_3$  it may be possible to assess the roles of N form and pH in modifying epiphytic community composition.

Should the factor determining the community composition of cryptogamic species be clearly identified as being N rather than pH then the finding would imply that the potential threat from  $\text{NH}_3$  pollution is not entirely restricted to the close proximity of point sources of the pollutant. Subsequently, this highlights potential concerns about N as a long-distance trans-boundary pollutant, as highlighted by van Herk *et al.* (2003<sup>a,b</sup>). This is especially important with regards to the mobile N forms  $\text{NH}_4^+$  and  $\text{NO}_3^-$  because they are the main N source for plants, including chlorolichens and bryophytes. A study was therefore undertaken to investigate the effect of wet deposited  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and PK on substratum pH and species colonisation at the Whim Moss experimental facility in the Scottish Borders (National Grid Reference NT 203 532). In addition to this, observations were made along the  $\text{NH}_3$  transect at the site, which included the experimental application of P and K, to support the findings at the wet deposition plots.

## **6.2 Methods**

### **6.2.1 Experimental Field Site**

Whim Moss (Figure 6.1) is a 0.5 ha field experimental site established to investigate the effects of eutrophication on an unmanaged ombrotrophic peat bog of the National Vegetation Classification M19a (Rodwell, 1991). The site

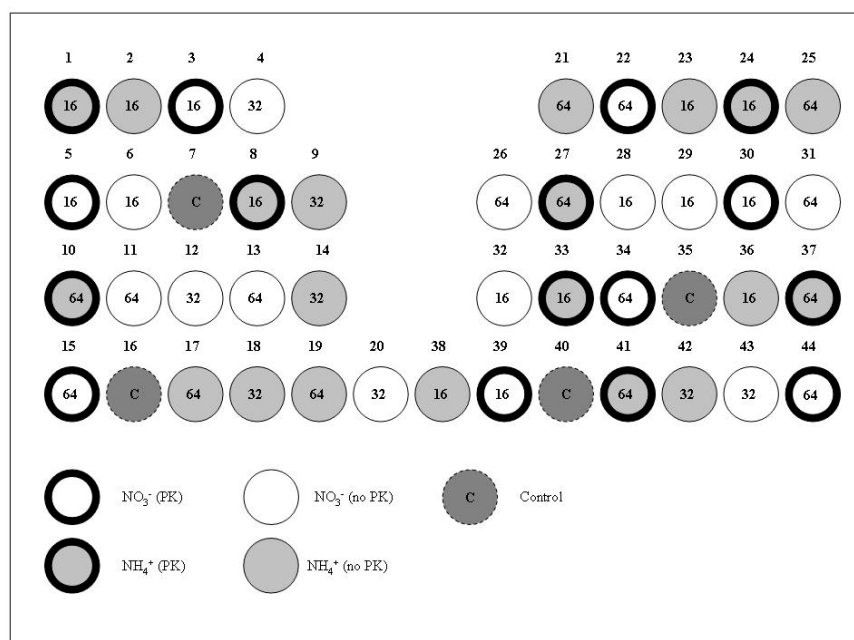
has been unmanaged for 60 years, receives a mean annual rainfall of 900 mm  $y^{-1}$  and a background N deposition of 8 kg  $ha^{-1} yr^{-1}$  (Sheppard *et al.*, 2004<sup>a</sup>). It is composed of two main sections accessible by boardwalks that investigate the effects of both dry and wet deposition respectively through the implementation of a fully automated system that has released N under appropriate wind and precipitation conditions since June 2002 (Sheppard *et al.*, 2004<sup>b</sup>). The dry deposition experiment is in the form of a 60 m transect where  $NH_3$  is released during dry conditions, when the wind speed is  $> 2.5 m s^{-1}$  and direction between 180 and 215° (Leith *et al.*, 2004).



**Figure 6.1** Aerial photograph of Whim Moss experimental plot, Scottish Borders (National Grid Reference NT 203 532). The plot comprises a central  $NH_3$  dry deposition transect with two lateral sections comprising the wet deposition  $NH_4^+$  and  $NO_3^-$  plots. Photograph courtesy of Ian Leith.

The wet deposition plots comprise a series of 44 randomised circular plots measuring 12.5  $m^2$  and spaced 3 m apart from each other (Sheppard *et al.*, 2004<sup>a</sup>). Four replicates of 11 different treatments exist in the wet deposition

plots (Figure 6.2). Six of the treatments had either  $\text{NH}_4^+$  (in the form of  $\text{NH}_4\text{Cl}$ ) or  $\text{NO}_3^-$  (as  $\text{NaNO}_3$ ) applied as a spray at three concentrations: 0.57 mM, 1.71 mM and 4 mM. Four of the remaining treatments had either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  applied at concentrations of 0.57 mM and 4 mM with PK added in the form of  $\text{K}_2\text{HPO}_4$  at a N:P ratio of 14:1. The remaining four plots were set aside as controls, where de-ionised water was applied as a spray during periods of treatment application.



**Figure 6.2** Plan of the wet deposition plots at Whim Moss. Numbers in the circles indicate estimated total deposition of either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  applied to each plot in  $\text{kg ha}^{-1} \text{y}^{-1}$ . The control plots (C) received background N deposition only ( $8 \text{ kg ha}^{-1} \text{y}^{-1}$ ).

Treatment sprays were applied during wet weather coupled with wind speeds  $< 5 \text{ m s}^{-1}$  originating from a direction between  $180$  and  $215^\circ$  (Leith *et al.*, 2004). The climatic conditions at the site meant the depositional values for the control, 0.57, 1.71 and 4 mM N concentrations plots equated to ca. 8, 16, 32

and  $64 \text{ kg ha}^{-1} \text{ y}^{-1}$  respectively, taking into account the natural background deposition of  $8 \text{ kg ha}^{-1} \text{ y}^{-1}$  (Sheppard *et al.*, 2004<sup>b</sup>).

### **6.2.2 Surface pH Measurements of Boardwalk Timber and *Calluna vulgaris* Stems**

The surface pH of the boardwalks was measured *in situ* using a BDH Gelplas double junction flathead electrode calibrated to pH 4.01 and 7.01. Three pH measurements were taken within each plot on the windward (south-facing) side of the boardwalks between the 20/05/2008 and the 02/06/2008. The methodology followed that of Schmidt (2000), which involved adding a piece of silica tubing over the flathead electrode, leaving a 2 mm exposed edge of silica at the end of the electrode head, that was used to hold a solution of KCl. One modification to this method was made, whereby no notch was cut into the silica tubing to insert the solution. This was because initial trials found that the silica tubing was prone to splitting along the cut notch with use. To compensate for this a piece of uncut silica tubing was applied and four drops of 0.25 M KCl solution placed into the 1 mm deep cup formed by the tubing around the head of the flat-head electrode with a pipette. The flat-head electrode was quickly and carefully placed against the boardwalk panels for ca. 3 minutes to measure substratum pH. The first boardwalk measurement in each plot was taken 1.4 m east of the plot centre, and subsequent measurements taken at 0.2 m intervals away from the centre point. This was to offset the effect of the prevailing wind at each plot.



The boardwalk pH along the dry  $\text{NH}_3$  deposition transect was measured on 12/08/2008. Measurements were taken from the west-facing panels of the central boardwalk of the  $\text{NH}_3$  transect, which maximised  $\text{NH}_3$  deposition driven by the prevailing wind. The central boardwalk section of the  $\text{NH}_3$  transect (Figure 6.1) was selected as it was less obscured by vegetation, than other boardwalk sections. Boardwalk pH was recorded at nine points along the  $\text{NH}_3$  transect. Measurements were made 4, 8, 12, 16, 20, 24, 32, 48 and 60 m. This coverage aimed to maximise transect coverage whilst ensuring each recording was taken at a point where Adapted Low-cost Passive High-Absorption (ALPHA) samplers had previously been installed, thus enabling comparative analysis of boardwalk pH values with recorded  $\text{NH}_3$  concentrations. Three replicate pH measurements were taken at each point along the  $\text{NH}_3$  transect. The first pH measurement at each point was taken perpendicular to each ALPHA sampler, and the remaining two taken 0.2 m either side of the first measurement.

The method for measuring the bark pH of dead *Calluna vulgaris* stems in the wet deposition plots and along the  $\text{NH}_3$  transect followed that of Kermit & Gauslaa (2001). Three stems 3-5 mm in diameter were collected from within each wet deposition treatment plot and  $\text{NH}_3$  transect point between 18/08/2008 and the 22/08/2008. These were air-dried and stored in paper bags for two weeks at room temperature (ca. 20°C) prior to analysis.

Stems were cut into 6 cm lengths, and the exposed ends sealed with paraffin wax. Each twig was soaked in 6 ml of 25 mM KCl solution for one hour in

sealed tubes to prevent atmospheric CO<sub>2</sub> ingress into the solutions. Each tube was frequently agitated to promote ionic exchange between bark and solution along the twig length. After one hour, each twig was removed and the pH of the solution measured using the same flathead electrode as described above. Mean values of *Calluna vulgaris* stems were then calculated for each wet deposition treatment plot and dry deposition recording point as a representative estimate of stem pH.

### **6.2.3 Epiphytic Cryptogam Species Composition**

Lichens and bryophytes growing on the *C. vulgaris* stems and boardwalks in and around each plot and on at each sampling point on the NH<sub>3</sub> transect were identified to species level following the nomenclatures of Smith *et al.* (2009) for lichens and Smith (2004) for bryophytes. Each species identified was placed into one of three ecological classes: nitrophobic, intermediate and nitrophilic. The basis of this grouping followed lichen species categorisation into either nitrophytes or acidophytes, as previously described by van Herk (1999) and van Herk *et al.*, (2002). Species not previously categorised as either acidophytic or nitrophytic were assumed to be intermediate for the purposes of this study. The total number of species at each plot was recorded for statistical analysis. *Hypogymnia physodes* and *H. tubulosa* were recorded as *Hypogymnia* agg. because a number of recorded thalli were too underdeveloped to confidently determine at the specific level. Both species are considered to be acidophytic by van Herk (1999), and both were identified as nitrophobes in the indicator species analysis (see Chapter 5). The nitrophilic species *Physcia adscendens* and *P. tenella* were recorded as *Physcia* agg. for the same reason.

#### **6.2.4 PK Application Experiment along the NH<sub>3</sub> Transect**

To investigate the potential influence of P and K addition further, PK was applied to the boardwalks along the NH<sub>3</sub> transect that were not exposed to PK as some of the wet deposition plots were (see Section 6.2.1). This experiment aimed to help address two matters; first, whether PK is the limiting factor at Whim Moss, and second, what the relative importance of substratum pH and [NH<sub>3</sub>] values had on species composition.

At the nine established survey points on the NH<sub>3</sub> transect (see Section 6.2.2), the end 7 cms of four 100 x 15 x 2.5 cm untreated *Larix* planks in the central boardwalk were treated with 20 ml of 0.29 mM K<sub>2</sub>HPO<sub>4</sub>. Treatments were applied manually using a separate brush on each transect point to limit potential cross-contamination with ascospores, isidia or soredia. All K<sub>2</sub>HPO<sub>4</sub> applications were undertaken after a minimum of three days dry weather to ensure sufficient absorption. Treatment continued for a 12-month period commencing 30/01/2009, and boardwalks re-surveyed for epiphytic growth on 06/06/2011. Ammonia values for each of the nine transect points were measured using ALPHA samplers (see Section 2.6), and the data acquired courtesy of Ian Leith and Lucy Sheppard (CEH, Edinburgh). Mean NH<sub>3</sub> values for each point were calculated from measurements taken between January 2007 and December 2007 inclusive.

#### **6.2.5 Statistical Analysis**

Minitab v.15 and v.16 was used to perform all statistical analyses except the Chi-squared ( $\chi^2$ ) Goodness of Fit, which was calculated manually. All

parametrically tested data were checked for normality and equal variance prior to analysis, and  $\log_{10}$  transformations applied where appropriate. The effect of N on substratum pH was tested using a one-way ANOVA, and supported non-parametrically using Kruskal-Wallis and Mann-Whitney U-Tests. This was done because of concerns arising from the low sample size ( $n = 4$ ) in the wet deposition plots, and subsequent risk of a type I error (Dytham, 2003). A PPMCC was undertaken on the  $\text{NH}_3$  transect data to identify relationships between substratum pH and distance from the  $\text{NH}_3$  source. Analyses investigating the influences of PK addition, N type and substratum pH on species composition was undertaken using  $\chi^2$  Goodness of Fit. Observations and measurements were made for the N treatments applied with 1.71 mM concentrations at Whim Moss, but analyses of these plots was excluded in all cases because of the lack of corresponding PK treatments.

## **6.3 Results**

### **6.3.1 Effect of N on Substratum pH**

Mean pH of the untreated *Larix* boardwalks (pH 3.56) in control plots receiving distilled water, was significantly more acidic than the *C. vulgaris* stems (pH 5.01) (2 sampled *t*-test;  $n = 4$ ;  $df = 3$ ; *t* value 5.58;  $P = 0.011$ ). Application of the different N forms within the wet deposition plots significantly influenced the mean pH of both the surrounding boardwalks, and the dead *C. vulgaris* stems (Table 6.1). This resulted in a polarising effect on substratum pH wherein it became more acidic with  $\text{NH}_4^+$  and less acidic with  $\text{NO}_3^-$  application relative to the control plots. It was also noted that the addition

of P and K increased the effect that N addition had on substratum pH. This was particularly noticeable in the 4 mM treatment plots (Figure 6.3).

The findings of a one-way ANOVA were supported by the non-parametric analysis for each treatment on both the boardwalks (Kruskal-Wallis;  $n = 4$ ;  $df = 1$ ;  $P = 0.001$ ), and *C. vulgaris* stems (Kruskal-Wallis;  $n = 4$ ;  $df = 1$ ;  $P = 0.003$ ). The non-parametric analysis also confirmed that substratum pH changed the most dramatically in the 4 mM  $\text{NO}_3^-$  (PK) and  $\text{NH}_4^+$  (PK) treatments. Median pH values at the 4 mM  $\text{NH}_4^+$  (PK) plots was 3.28 and 4.24 for the boardwalks and *C. vulgaris* stems respectively, and were significantly (Mann-Whitney;  $P < 0.05$ ) more acidic than those in the majority of other treatment types (Table 6.2). The 4 mM  $\text{NO}_3^-$  treatments mirrored these findings, with the boardwalks (median pH 4.26) and *Calluna* stems (median pH 5.66) being significantly (Mann-Whitney;  $P < 0.05$ ) less acidic than most of the other treatment plots (Table 6.2).

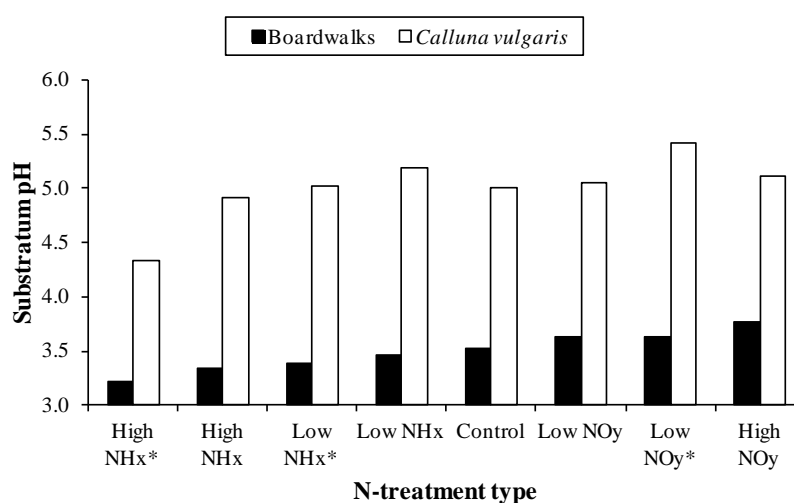
**Table 6.1** Substratum pH differences between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treated *Larix* boardwalks (a) and *Calluna vulgaris* (b) stems at Whim Moss using a one-way ANOVA.

a)	Source	DF	SS	MS	F	P
	N-type	1	2.2754	2.2754	23.53	< 0.001
	Error	38	3.674	0.0967		
	Total	39	5.9494			
	S = 3109 R-Sq = 38.25% R-Sq(adj) = 36.62%					
	Branch Age Class	n	Mean pH	SD		
	$\text{NH}_4^+$	20	3.37	0.1874		
	$\text{NO}_3^-$	20	3.84	0.3978		
	Pooled SD			0.3109		

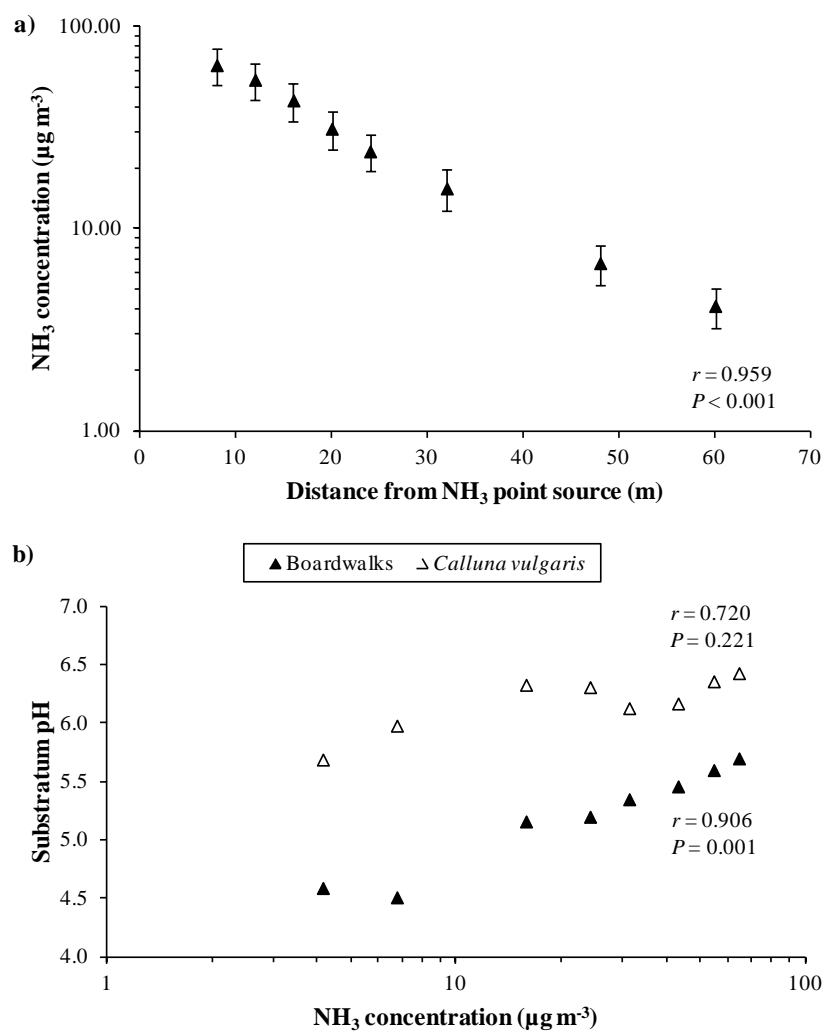
b)	Source	DF	SS	MS	F	P
	N-type	1	2.045	2.045	16.15	< 0.001
	Error	38	4.811	0.127		
	Total	39	6.856			
	S = 0.3558 R-Sq = 29.82% R-Sq(adj) = 27.98%					
	Branch Age Class	n	Mean pH	SD		
	$\text{NH}_4^+$	20	4.94	0.3478		
	$\text{NO}_3^-$	20	5.40	0.3637		
	Pooled SD			0.3558		

A strong negative logarithmic relationship was seen between  $[\text{NH}_3]$  values and distance from point source (Figure 6.4a), highlighting the rapid dispersal of the

gas. A clear trend existed in substratum pH of both boardwalks and *C. vulgaris* stems along the dry deposition  $\text{NH}_3$  transect wherein acidity was negatively associated with  $[\text{NH}_3]$  (Figure 6.4b), although the relationship for the *C. vulgaris* stems was not significant. Boardwalk pH values along the entire transect length were less acidic than those for unexposed boardwalks in the control wet deposition plots (see Section 6.3.1), with values ranging from pH 5.70 to 4.51 between 4-60 m from the point source. *Calluna vulgaris* stem pH also followed this trend with values ranging from 6.45 to 5.69 where, similarly, all values were less acidic than stems of *C. vulgaris* in the wet deposition control plots.



**Figure 6.3** Substratum pH values for *Larix* boardwalks (black) and *Calluna vulgaris* stems (grey) at the wet deposition treatment plots where either  $\text{NH}_4^+$  or  $\text{NO}_3^-$  was applied in concentrations of 0.57 mM (low) or 4 mM (high). Columns indicated (\*) represent treatments where P and K were added at a N:P ratio of 14:1.



**Figure 6.4** The logarithmic relationships between  $[\text{NH}_3]$  and (a) distance from point source, and (b) substratum pH along the  $\text{NH}_3$  transect at Whim Moss. Analyses were undertaken using Pearson's Product Moment Correlation Co-efficient.

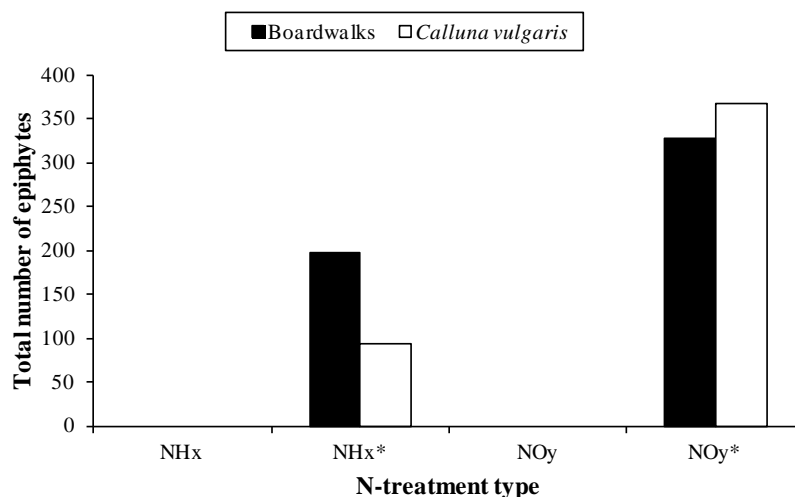
**Table 6.2** Between treatment differences in substratum pH at wet deposited plots at Whim Moss for boardwalks (plain text) and *C. vulgaris* stems (italics). Bold type indicates significant differences at the  $P < 0.05$  level (Mann-Whitney U-test).

	4 mM $\text{NH}_4^+$ (PK)	4 mM $\text{NH}_4^+$	0.57 mM $\text{NH}_4^+$ (PK)	0.57 mM $\text{NH}_4^+$	Control	0.57 mM $\text{NO}_3^-$	0.57 mM $\text{NO}_3^-$ (PK)	4 mM $\text{NO}_3^-$	4 mM $\text{NO}_3^-$ (PK)
4 mM $\text{NH}_4^+$ (PK)	-	0.6650	0.3123	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>
4 mM $\text{NH}_4^+$	0.0606	-	1.0000	0.6650	0.665	0.3123	0.3123	0.1124	<b>0.0304</b>
0.57 mM $\text{NH}_4^+$ (PK)	0.0606	0.6650	-	0.4705	0.3123	0.3040	0.1124	0.1124	<b>0.0304</b>
0.57 mM $\text{NH}_4^+$	<b>0.0304</b>	0.1489	0.4705	-	0.4705	0.1124	0.3123	0.1124	<b>0.0304</b>
Control	<b>0.0304</b>	1.0000	0.8852	0.3123	-	0.6650	0.4705	0.1124	<b>0.0304</b>
0.57 mM $\text{NO}_3^-$	<b>0.0304</b>	0.8852	0.8852	0.3123	0.6650	-	0.8852	0.3123	<b>0.0304</b>
0.57 mM $\text{NO}_3^-$ (PK)	<b>0.0304</b>	0.0304	0.0304	0.0304	0.1939	0.0606	-	0.3123	<b>0.0304</b>
4 mM $\text{NO}_3^-$	0.0606	0.3123	0.4705	0.6650	0.6650	0.4705	0.5637	-	<b>0.0304</b>
4 mM $\text{NO}_3^-$ (PK)	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>	<b>0.0304</b>	0.0606	<b>0.0304</b>	0.2482	0.1939	-

### 6.3.2 Effect of PK Addition on Species Composition

One bryophyte and 11 lichen species (including *Hypogymnia physodes*, *H. tubulosa*, *Physcia adscendens* and *P. tenella*, which were grouped at the generic level as per Section 6.2.3) were recorded growing on the *Larix* boardwalks and *C. vulgaris* stems. Six were classified as being nitrophobic, three intermediate and three nitrophilic. The boardwalks supported 527 individuals comprised of eight species, and the *C. vulgaris* stems 444 individuals of 10 species. There was a clear pattern of cryptogamic plant distribution with occurrence almost exclusively restricted to the PK treated plots (Figure 6.5). Only one treatment plot not treated with PK showed signs of epiphytic growth. This was a 1.71 mM  $\text{NO}_3^-$  (no PK) where a single thallus of *Usnea* sp. had established on the boardwalks. However, as the 1.71 mM treatment plots were not included in the analyses (see Section 6.2.1), this was not considered.





**Figure 6.5** Total number of epiphytes recorded on boardwalks and *C. vulgaris* stems at Whim Moss either with (\*) or without PK.

Statistical analysis was undertaken using a  $\chi^2$  Goodness of Fit comparing the total number of cryptogamic species in treatments with P and K applied against those without clearly indicated a significant bias in cryptogam distribution in favour of PK treated plots ( $\chi^2$  Goodness of Fit;  $n = 988$ ;  $df = 1$ ;  $\chi^2_1 = 944$ ;  $P < 0.001$ ). This was reflected on both the boardwalks ( $\chi^2$  Goodness of Fit;  $n = 527$ ;  $df = 1$ ;  $\chi^2_1 = 944$ ;  $P < 0.001$ ) and *C. vulgaris* stems ( $\chi^2$  Goodness of Fit  $n = 461$ ;  $df = 1$ ;  $\chi^2_1 = 461$ ;  $P < 0.001$ ). As a result of this finding all further statistical analyses involving the effect of N on epiphytes at the wet deposition plots focussed exclusively upon PK treated plots. In addition to this, observations for boardwalks and *C. vulgaris* stems were combined in some subsequent analyses of wet deposition data in this Chapter due to the limited number of replicates ( $n = 4$ ) available for analysis.

Further evidence that PK was a limiting nutrient source was shown by the positive results obtained on the NH<sub>3</sub> transect. Algal and lichen growth was observed on all PK treated boards, while no growth was visible on either the untreated sections of the fertilised boards or adjacent sections. Identification of species was not possible in almost all cases as thalli were very small and in the early stages of development. Consequently, a morphological species concept was established to group organisms. Although this technique was very crude, it identified a change in species distribution along the NH<sub>3</sub> transect based upon distance from point source (Table 6.3). Only two individual lichen thalli were identifiable to generic level (*Hypogymnia* sp. and *Xanthoria* sp.).

**Table 6.3** Number of morphologically similar species on PK treated *Larix* boardwalks at different survey points on the NH<sub>3</sub> transect.

Species	Distance from NH <sub>3</sub> point source (m)								
	4	8	12	16	20	24	32	48	60
Species 1	5	6	6	0	0	0	0	0	0
Species 2	0	0	6	6	6	0	0	0	0
Species 3	0	0	0	3	0	0	0	0	0
Species 4	0	0	0	0	1	0	0	0	0
<i>Xanthoria</i> sp.	0	0	0	0	1	0	0	0	0
Species 5	0	0	0	3	2	2	2	1	0
Species 6	6	0	0	4	6	6	6	6	6
Species 7	0	0	0	0	0	1	3	6	5
Species 8	0	0	0	0	0	3	1	0	2
<i>Hypogymnia</i> sp.	0	0	0	0	0	0	0	0	1

### 6.3.3 Effect of N Form on Species Composition

Independent •<sup>2</sup> Goodness of Fit analysis of nitrophobes, intermediates and nitrophiles growing on the boardwalks and *C. vulgaris* stems combined at the wet deposition treatment plots indicated that N form was important in the distribution of the three ecological classes. Abundance of intermediate and

nitrophilic species was significantly greater in  $\text{NO}_3^-$  treated plots, and nitrophobic species in the  $\text{NH}_4^+$  plots (Figure 6.6; Table 6.4). In the case of the nitrophilic species, no thalli were observed in any of the  $\text{NH}_4^+$  treated plots.

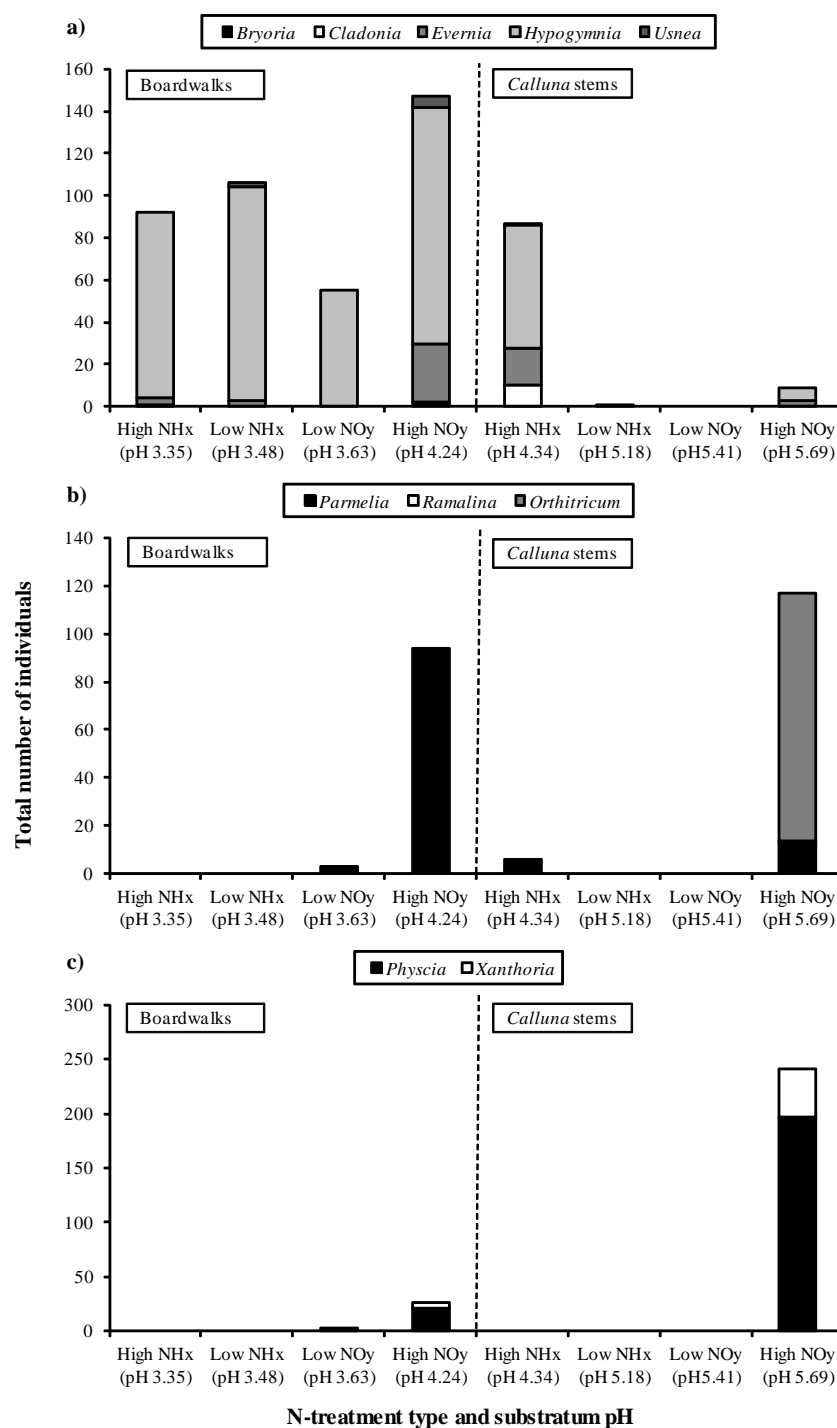
The apparent preference in N form of individual lichen species largely reflected the N-response classes in which they were categorised (Table 6.4). The nitrophobic *Evernia prunastri* was an exception, its abundance being evenly distributed between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  plots. This observation was supported statistically through  $\chi^2$  analysis (Table 6.4). *Orthotrichum* sp. was the sole bryophyte recorded in the plots, and had an atypical distribution. Although the moss is classified as an intermediate between nitrophobic and nitrophilic (Figure 6.6b), at Whim it was restricted to *C. vulgaris* stems subjected to high concentrations of  $\text{NO}_3^-$  and with high pH values (mean = 5.69) (Figure 6.6).

**Table 6.4** Wet deposited N-form preference of lichens at Whim Moss. Assessment of the three N-response classes (a), and the five most abundant lichen species (b) growing on both substrata in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  plots, was undertaken using a Chi-squared Goodness of Fit analysis of total thalli counts.

a)					
N-response Class	<i>n</i>	df	$\chi^2_1$	<i>P</i>	
Nitrophobes	497	1	11.32	< 0.001	
Intermediates	117	1	94.23	< 0.001	
Nitrophiles	270	1	270.00	< 0.001	

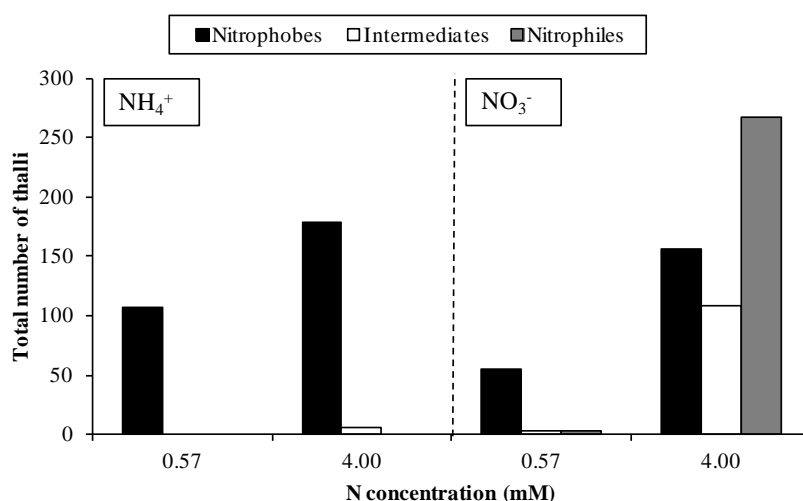
b)						
Lichen Species	N-response Class	<i>n</i>	df	$\chi^2_1$	<i>P</i>	
<i>Evernia prunastri</i>	Nitrophobe	55	1	0.89	> 0.05	
<i>Hypogymnia</i> agg.	Nitrophobe	421	1	13.36	< 0.001	
<i>Parmelia sulcata</i>	Intermediate	116	1	93.24	< 0.001	
<i>Physcia</i> agg.	Nitrophile	218	1	218.00	< 0.001	
<i>Xanthoria parietina</i>	Nitrophile	52	1	52.00	< 0.001	



**Figure 6.6** Total number of individual lichens and bryophytes on boardwalks and *C. vulgaris* stems at Whim Moss in the three N-response classes: nitrophobes (a), intermediates (b) and nitrophiles (c).

### 6.3.4 Effect of NPK Concentration on Lichen Abundance

Total numbers of lichen thalli for the three N-response classes were significantly greater in the 4.0 mM than the 0.57 mM treatments for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the wet deposition plots (Figure 6.7). Although all lichen classes, and the respective species comprising them, increased in abundance in response to increased  $\text{NO}_3^-$  and P, the rate of increase differed between nitrophobes and the other two classes. Abundance of nitrophiles and intermediates increased from 3 to 267 (8900%) and 3 to 108 (3600%) respectively, considerably more than the nitrophobic species, which increased from 55 to 156 individuals (283.6%). Conversely, only the nitrophobic species showed a positive response to increased NPK levels in the  $\text{NH}_4^+$  treated plots (Figure 6.7).



**Figure 6.7** Total number of lichen thalli recorded on both the boardwalks and *Calluna vulgaris* stems in the wet deposition plots treated with P and K.

### 6.3.5 Effect of Substratum pH on Lichen Species Composition

In order to investigate the effect of substratum pH on lichen abundance, the results for *C. vulgaris* stems and *Larix* boardwalks were aggregated. This also resulted in data for the  $\text{NH}_4^+$  (PK) and  $\text{NO}_3^-$  (PK) treatments being mixed, thus helping to reduce the confounding influence of N form that was previously shown to affect lichen distribution (Sections 6.3.3-6.3.4). Data for the total numbers of individuals in the three N-response classes were then summed for surfaces with pH values less than or greater than 4.40, a value that roughly split the dataset into two equal halves.

A significant bias towards a specific substratum pH was observed for both nitrophobes and nitrophiles (Table 6.5). Nitrophobic species were 12.5 times as abundant on substrata with a  $\text{pH} < 4.40$ , whereas nitrophiles were three times more abundant on substratum with a  $\text{pH} > 4.40$  (Figure 6.8). No significant difference was observed for the intermediate species (Table 6.5). Data for individual lichen species from each of the three ecological classes replicated the findings at the N-response class level (Table 6.5).

Furthermore, the *Xanthoria* thallus was found growing at the 20 m and the *Hypogymnia* at the 60 m points along the  $\text{NH}_3$  transect (Table 6.3). Boardwalk surface pH and  $[\text{NH}_3]$  values at the 20 m point were 5.35 and  $31.27 \mu\text{g m}^{-3}$  respectively, which support both the view of the importance of higher pH values and N concentrations on nitrophilic species. Values of pH and  $[\text{NH}_3]$  at the 60 m point were 4.51 and  $3.95 \mu\text{g m}^{-3}$  respectively. Although substratum pH was sufficiently close to suitable conditions for nitrophobic species (Figure

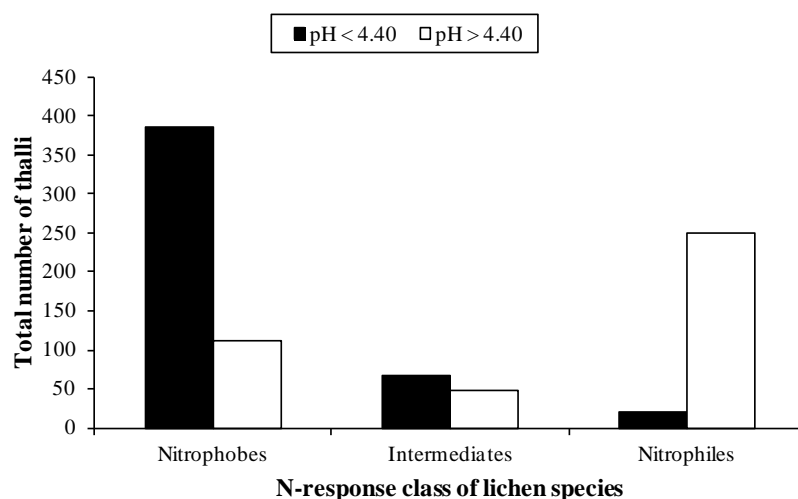
6.6a),  $[\text{NH}_3]$  values were above the  $1 \mu\text{g m}^{-3}$  CLE set for sensitive habitats and species (Cape *et al.*, 2009).

**Table 6.5** Relationships between substratum pH and number of lichen thalli within the three N-response classes (a) and for individual species (b). A Chi-squared Goodness of Fit indicated that the indicator species groups (nitrophobes and nitrophiles) displayed a significant preference for  $\text{pH} < 4.40$  and  $\text{pH} > 4.40$  respectively (Figure 6.8). The same trend was seen at the species level.

a)	N-response Class	<i>n</i>	df	$\chi^2_1$	<i>P</i>
	Nitrophobes	497	1	152.16	< 0.001
	Intermediates	117	1	3.09	> 0.05
	Nitrophiles	270	1	270.00	< 0.001

b)	Lichen Species	N-response Class	<i>n</i>	df	$\chi^2_1$	<i>P</i>
	<i>Evernia prunastri</i>	Nitrophobe	55	1	8.02	< 0.01
	<i>Hypogymnia</i> agg.	Nitrophobe	421	1	137.96	< 0.001
	<i>Parmelia sulcata</i>	Intermediate	116	1	3.45	> 0.05
	<i>Physcia</i> agg.	Nitrophile	218	1	169.10	< 0.001
	<i>Xanthoria parietina</i>	Nitrophile	52	1	27.77	< 0.001

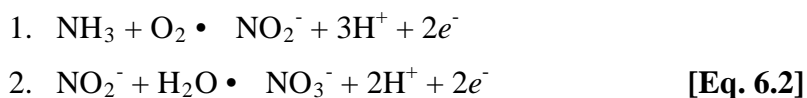
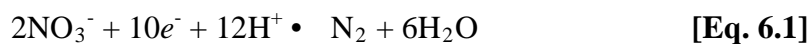


**Figure 6.8** Number of lichen thalli in each of the three N-response classes on boardwalks and *C. vulgaris* stems at Whim Moss with a surface pH less than or greater than 4.40.

## 6.4 Discussion

### 6.4.1 N Effects on Substratum pH

NH<sub>3</sub> pollution raised the pH of the substratum along the NH<sub>3</sub> transect, which was consistent with established findings (Fрати *et al.*, 2007; van Dobben & ter Braak, 1998). The weaker relationship between [NH<sub>3</sub>] and pH of *C. vulgaris* stems might be explained by effects resulting from temporal variation in soil-nutrient uptake by the plant, and its subsequent deposition on the stem, which would be determined by time of stem death. The polarised changes in substratum pH in response to NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> treatment was potentially due to biological processes rather than a direct chemical effect. One possible explanation is that a net uptake of H<sup>+</sup> from the substratum occurred through the redox reaction of NO<sub>3</sub><sup>-</sup> respiration through denitrifying bacteria (Eq. 6.1). Net acidification of substrata in the NH<sub>4</sub><sup>+</sup> treated plots may be caused by the application of N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; however, it is possible that the nitrification process may play a role (Eq. 6.2). This is a hypothetical explanation, but a simple experiment investigating the microbial communities present on the boardwalks would help test the validity of this statement, as *Pseudomonas* would be expected to be more prevalent at the NO<sub>3</sub><sup>-</sup>, and *Nitrobacter* and *Nitrosomonas* at the NH<sub>4</sub><sup>+</sup> plots.





#### **6.4.2 PK Effects on Species Composition**

All of the most abundant species recorded at Whim Moss were found growing on both substrata, and this corroborated independent personal field observations. Consequently, any complications relating to a specifically corticolous or lignicolous habit of the lichens recorded was negated. The absence of lichen colonisation in wet deposition plots not treated with PK indicated the importance of PK to lichen establishment. This was supported by the survey of the  $\text{NH}_3$  transect post-PK application and provided evidence that P was the limiting factor to lichen development at Whim Moss where N application occurred, following Leibig's Law of the Minimum. It also concurs with findings of Benner & Vitousek (2007) that P fertilisation of forest soils resulted in a dramatic increase in epiphytic lichen abundance, and previous fertilisation experiments on forest bryophytes (Dirske & Martakis, 1992).

#### **6.4.3 Influence of N Form on Lichen Species Composition**

Assessments of the influence of N form for both substratum types combined showed that there was a clear preference for  $\text{NO}_3^-$  in the nitrophilic and intermediate lichens, but the nitrophobic species were abundant in both N treatment plots, and significantly so in the case of *Evernia prunastri*, where substratum pH was  $< 4.40$  (Figure 6.6; Table 6.4). The presence of nitrophobes at both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  treated plots suggests they probably can effectively utilise both N forms, and that restrictions to their presence is due to other factors, such as surface pH or P limitation. Conversely, the absence of nitrophiles in plots where  $\text{NH}_4^+$  was applied implies uptake of reduced N for effective growth and development by these species is much more limited.

Earlier experiments by Gaio-Oliveira *et al.* (2004) have shown the nitrophile *Xanthoria parietina* is tolerant of very high  $\text{NH}_4^+$  levels (i.e. treatments of 0.04 to 0.69 M  $\text{NH}_4\text{Cl}$ ), and that saturation of the cation exchange capacity (CEC) of the thallus was one likely explanation for this (Gaio-Oliveira *et al.*, 2001). Differences between the CEC of nitrophobes and nitrophiles would possibly explain the observed species split at the wet deposition plots. No investigations were undertaken to identify potential CEC differences, but tentative evidence pointing to this exists in the thallus surface pH experiment (Chapter 6), where the nitrophobic *Evernia prunastri* displayed a more acidic thallus pH than that of *X. parietina* across all survey sites.

#### **6.4.4 Influence of Substratum pH on Lichen Species Composition**

Nitrophobic lichens were more abundant than nitrophiles or intermediates on the more acidic substrata. There was a clear pattern in which nitrophobes dominated at  $\text{pH} < 4.40$  and nitrophiles at  $\text{pH} > 4.40$  (Figure 6.8). Although the substratum pH split homogenised the N treatment data, it did not effectively split the substratum types. However, even though only one of the *C. vulgaris* treatments had a mean substratum  $\text{pH} < 4.40$ , the marked split in nitrophobic and nitrophilic species at opposing ends of the pH range, together with the strong presence of nitrophobes on the boardwalks of the high  $\text{NO}_3^-$  treatment plots, supports the notion of pH playing a key role in lichen colonisation. This is further supported from the observations along the  $\text{NH}_3$  transect where the initial investigation has highlighted a potential community composition split along the boardwalk transect with potentially more nitrophilic species closer to

the point source where substratum pH was higher, and nitrophobic species located at the distal end where the pH was more acidic.

#### **6.4.5 PK Application Experiment on the NH<sub>3</sub> Transect**

Unfortunately it is not possible to draw definitive conclusions from the addition of PK to the NH<sub>3</sub> transect with respect to the influence of either N or substratum pH on lichen colonisation. This is due to a lack of data and insufficient replication. However, a clear partitioning of morphospecies was seen along the transect, and PK was identified as a limiting factor in all the locations at Whim Moss where N alone is being applied. Finally, although substratum pH was higher on both boardwalks and *C. vulgaris* stems along the entire length of the NH<sub>3</sub> transect compared to background values (control measurements at wet deposition plots), surface pH fell to values as low as ca. 4.50 on boardwalks at the distal end of the transect. This was close to the preferred pH range for nitrophobes, as determined in the wet deposition plots (Figure 6.6a), and was also where the only identifiable thallus of *Hypogymnia* colonised along the NH<sub>3</sub> transect post-PK treatment (Table 6.3). This, together with the non-specific preference for N form displayed by some nitrophobes on substratum with a pH < 5.00 (Figure 6.6) offers tentative evidence for the importance of pH in early lichen colonisation and/or development.

#### **6.4.6 Critique**

Two major issues cast uncertainty on the results of this study. The first is that a fundamental disparity in pH measurements between boardwalks and *C. vulgaris* stems resulting from the different surface area to KCl volume ratios

for the two substrata caused by the different methods used (see Section 6.2.1-6.2.3). The second issue was related to complications in the statistical analyses due to limited replication ( $n = 4$  for wet deposition plots;  $n = 1$  for dry deposition transect) and the high number of zero values for cryptogam counts. The latter problem might be offset by undertaking an ordination analysis, however, the statistical package required for this was not available at the time of data analysis. Consequently, Chi-squared analyses were undertaken which were not as integrative a form of analysis and did not eliminate the issue of co-variance. Despite this, the findings of the Chi-squared analyses did appear to corroborate previous findings relating to the species composition at plots and pH (de Bakker, 1989; van Dobben *et al.*, 2001; van Herk, 1999).

## Chapter 7. Lichen Phosphatase Activity and Chemistry

### 7.1 Introduction

A majority of epiphytic lichens are associated with nutrient poor habitats, and many have developed mechanisms to optimise nutrient acquisition as a result. This project has identified groups of lichens with apparent contrasting responses to N pollution, i.e. nitrophobes and nitrophiles. The distinct ecologies of several species in these groups has been recognised throughout Europe (van Herk, 1999; Wolseley *et al.*, 2005<sup>a</sup>). However, there is no evidence to date of any physiological difference to explain these contrasting ecologies.

One possible physiological difference between nitrophobic and nitrophilic species is the rate of phosphomonoesterase (PME) activity. PME is one of a number of phosphatase enzyme classes, including phosphodiesterases and phosphotriesterases, which catalyse the hydrolysis of phosphoric acid esters by breaking the R-O-P link to release an organic moiety and orthophosphate (Cembella *et al.*, 1984; Jansson *et al.* 1988). Phosphorous is only present in trace concentrations in atmospheric deposits, and only the inorganic forms (e.g.  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$  and  $\text{PO}_4^{3-}$ ) are typically available for uptake. The ability of PME to break up organic P has resulted in it being proposed as a means of P acquisition by lichens (Hogan, 2009), and past studies provide indirect evidence suggesting lichens can access biologically unavailable organic P in this manner (Lane & Puckett, 1979; Le Seur & Puckett, 1980). Furthermore, PME activity has been shown to increase linearly with N

deposition as a result of a shift in the N:P ratio in the lichen thallus from one of N-limitation to one of P-limitation (Hogan *et al.*, 2010<sup>a</sup>; Lane & Puckett, 1979; Makkonen *et al.*, 2007).

Crittenden *et al.* (in prep) identified differences in both the PME activity and optimum pH values of the nitrophobic *Usnea sphacelata* and nitrophilic *Xanthomendosa borealis*, thus providing preliminary evidence of a possible physiological difference between nitrophobes and nitrophiles. Furthermore, Hogan (2009) found that PME activity is greatest for species possessing an acid pH optima for PME activity, and Paul *et al.* (2009) have suggested that pH preferences of epiphytic lichens may be partially explained by pH dependant P uptake. This is supported by Hogan *et al.* (2010<sup>b</sup>) who found correlations between PME activity and pH. This discovery might indicate that PME activity is influenced by pH, which in turn is affected by [NH<sub>3</sub>].

Intracellular pH changes in vascular plants have been linked to NH<sub>3</sub> (Yin *et al.*, 1998), and the subsequent use of foliar pH as a trait to predict interspecific variation proposed (Cornelissen *et al.*, 2006). Lichens are potentially more susceptible to this type of NH<sub>3</sub> driven change due to their lack of a cuticle, and preliminary investigations measuring lichen thallus surface pH suggests that nitrophobes and nitrophiles do possess different pH values (Leith, pers comm.. 2008). Consequently, thallus surface pH measurements could be useful in two ways. First, it could be used as a simple means of identifying nitrophobic and nitrophilic species. Second, intraspecific variation in thallus pH values could prove an alternative means of evaluating [NH<sub>3</sub>], one potentially more reliable

than the well documented relationship between  $[\text{NH}_3]$  and bark pH (Frati *et al.*, 2007; van Herk, 1999; Wolseley *et al.*, 2005<sup>a</sup>) as confounding factors such as past perturbation and leaching would be reduced.

The current programme of work provided an opportunity to make more detailed comparisons between nitrophobes and nitrophiles in terms of PME activity and thallus pH. This work consequently tested the following hypotheses:

1. PME activity increases with increased nitrophobicity.
2. Lichen thallus surface pH increases with increasing N tolerance.

In addition to this, preliminary data on thallus chemistry for representative nitrophobes and nitrophiles are presented.

## **7.2 Methods**

### **7.2.1 Collection and Pre-treatment of Lichen Samples**

Details of collection sites are given in Table 7.1. All collected lichen specimens were air-dried for 24 hours and placed in paper packaging for storage purposes. Specimens used in the preliminary thallus pH measurements (see Section 7.2.3) and lichen thallus chemistry analysis (Section 7.2.5) were stored in the dark at room temperature (ca. 15 °C). Material for all other experiments was stored at -20 °C for a period not exceeding six weeks. Where required, re-hydration was undertaken by placing specimens in a water saturated atmosphere (over water in a dessicator) overnight in a plant growth room at 10°C and then lightly

sprayed with de-ionised water to ensure full hydration. Unless otherwise stated, rehydrated thalli were cleared of extraneous debris before analysis.

### **7.2.2 Measurement of Phosphomonoesterase Activity**

The terminal 5 mm of each lichen thallus was cut off with a razor blade, blotted with tissue to remove excess water and then added to 2.9 ml assay medium comprising simulated rainfall (150  $\mu$ M NaCl, 20  $\mu$ M MgSO<sub>4</sub>·7H<sub>2</sub>O, 15  $\mu$ M NH<sub>4</sub>NO<sub>3</sub>, 8  $\mu$ M CaCl<sub>2</sub>·2H<sub>2</sub>O, 5  $\mu$ M KNO<sub>3</sub>) adjusted to pH values ranging between 3.1-6.6 (Table 7.2) using 0.1 M citric acid and 0.1 M tri-sodium citrate buffer solutions following the method of Dawson *et al.* (1986) as described by Hogan (2009). The ionic composition of the assay medium aimed to simulate typical concentrations of the major ions present in UK rainfall as described by Hayman *et al.* (2004). Enzyme activity across the range of pH values was plotted for each species and the apparent optimum assay medium pH was then selected for subsequent interspecies comparisons of phosphatase activity.

The assay was initiated by addition of 0.1 ml 15 mM 4-nitrophenol phosphate (*p*NPP) solution (to yield a final concentration of 500  $\mu$ M *p*NPP in 3 ml) and the samples incubated in a water bath for 20 minutes at 15°C in the dark. The assay was terminated by removing 2.5 ml of the incubation medium, to which 0.25 ml of terminator solution (1 M NaOH, 27.5 mM EDTA, 0.55 M K<sub>2</sub>HPO<sub>4</sub>) was added and the optical density measured at 405 nm using a Nanodrop ND-1000 spectrophotometer. The assayed thallus tips were blotted dry, oven dried at 80°C for 24 hours and weighed on a Metler AE240 balance.



**Table 7.1** Collection details of lichen specimens used in the experiments investigating phosphomonoesterase activity, thallus pH and thallus chemistry, together with modelled NH<sub>3</sub> concentrations in air.

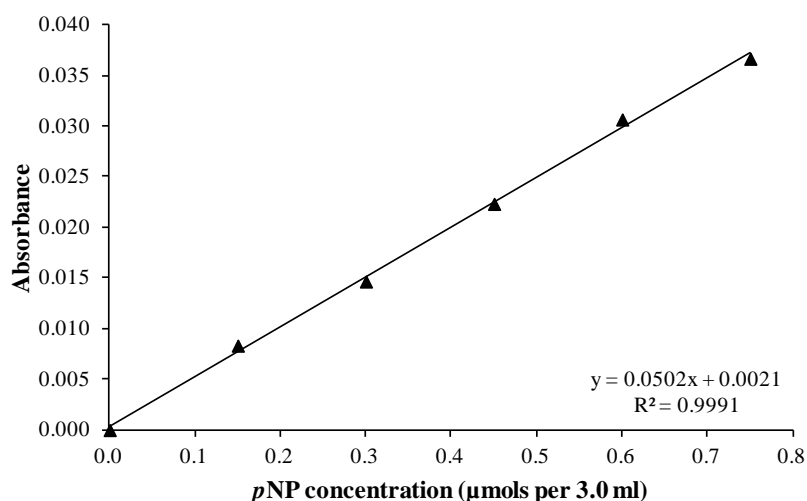
Site Name	National Grid Ref.	[NH <sub>3</sub> ] µg m <sup>-3</sup>	Lichen Species	Collection Date	Collector
<b>Phosphomonoesterase Measurement</b>					
Banchory	NO 677 986	0.18	<i>Evernia prunastri</i> and <i>Hypogymnia physodes</i>	27/09/2010	J.E.J. Lewis
Upper Derwent	SK 146 935	0.56	<i>Evernia prunastri</i> and <i>Platismatia glauca</i>	15/09/2010	J.E.J. Lewis & P.D. Crittenden
University Park	SK 542 384	1.85	<i>Phycia adscendens</i> , <i>Xanthoria parietina</i> and <i>Xanthoria polycarpa</i>	27/09/2010	J.E.J. Lewis & P.D. Crittenden
<b>Lichen Thallus Surface pH Measurement</b>					
Bretton Lakes	SE 282 123	0.94	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	10/12/2011	J.E.J. Lewis
Bush Estate	NT 245 635	1.49	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	08/12/2011	I.D. Leith
Redgrave and Lopham Fen	TM 053 802	2.79	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	02/12/2011	P. Lambley
Tycanol Wood	SN 090 366	1.94	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	28/05/2011	P.A. Wolseley
Yarner Wood	SX 786 789	0.54	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	10/12/2011	P.A. Wolseley
<b>Lichen Thallus Elemental Analysis</b>					
Brown Moss	SJ 563 390	3.03	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	14/07/2009	J.E.J. Lewis
Bush Estate	NT 245 635	1.49	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	24/05/2010	J.E.J. Lewis
North Wyke	SX 659 983	2.07	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	30/03/2009	J.E.J. Lewis & P.A. Wolseley
Wytham Wood	SP 462 082	1.30	<i>Evernia prunastri</i> , <i>Parmelia sulcata</i> and <i>Xanthoria parietina</i>	21/08/2009	J.E.J. Lewis

No lichen ( $N_L$ ) and no substrate ( $N_S$ ) controls were run in parallel with the assays to provide blank measurements and to check for anomalous effects. Any values obtained from the controls were factored in to the final calculation (Eq. 7.1). The assay was calibrated with nitrophenol ( $p$ NP) in assay medium containing terminator solution (Figure 7.1). Lichen PME activity was expressed as the quantity of substrate hydrolysed in mmols ( $S_H$ ) per unit dry weight (g,  $D_w$ ) per unit time (h) according to Eq. 7.1, where no lichen ( $N_L$ ) and no substrate ( $N_S$ ) are control values.

**Table 7.2** Buffered assay medium pH values used in PME activity analysis.

Assay medium	ml 0.1 M citric acid	19.5	18.2	8.2	6.85	5.9	4.95	3.5	2.1	0.8
	ml 0.1 M trisodium citrate	0.5	1.8	1.8	3.15	4.1	5.05	6.5	7.9	9.2
Medium pH		3.0	3.2	3.6	3.9	4.3	4.8	5.4	6.0	6.6

$$\text{PME activity (mmols g}^{-1}\text{)} = \frac{[(S_H - N_L - N_S) / 1000]}{D_w \times 1000 \times 3} \quad [\text{Eq. 7.1}]$$



**Figure 7.1** Relationship between concentration of  $p$ NP and absorbance (each point is a mean of three values).

### **7.2.3 Measurement of Lichen Thallus pH**

Two methods of measuring thallus pH were used. The first was based on that proposed by Schmidt *et al.* (2001) for measuring bark pH. It was used to compare the effects of de-ionised water or 0.25 mM KCl as wetting agents, and to investigate the effect of measurement time on the pH value recorded. A cleaned lichen thallus was placed in a Petri dish and 3 ml of either de-ionised water (pH 6.08 at 20°C) or 0.25 mM KCl (pH 5.86 at 20°C) was dispensed onto it and then the dish lid replaced to minimise CO<sub>2</sub> ingress. Sequential thallus pH measurements were recorded at six time intervals from 5 mins up to 24 h for the purposes of studying the effects of measurement time, and after 30 minutes for comparisons of de-ionised water versus KCl, using a Gelplas double junction flat-head electrode calibrated to pH 4.01 and 7.01 with buffer solutions.

The second method followed Hogan (2009). A re-hydrated lichen thallus was placed in a Petri dish and two microscope slides placed over the thallus 15 mm apart. The ends of the slides were wetted with de-ionised water and two further slides placed over the ends of the first two slides and 20 mm apart to form a fixed-area of contained thallus. De-ionised water was added to each thallus until a water film was visible. The flat-head pH electrode was then placed firmly in contact with the thallus, ensuring it was coupled to the thallus by a continuous layer of water, and the pH value recorded.

#### **7.2.4            Elemental Analysis of Lichen Thalli**

Samples were removed from branches, cleaned of extraneous debris, air-dried and stored as described in Section 7.2.1. Samples of individual species from each site were bulked together due to there being insufficient material available for independent analysis in a number of cases. The bulked specimens were ground using a Retsch® MM200 ball grinder and sent to CEH Lancaster for analysis using the method described by Allen (1989). Total N and P, expressed as percentage dry weight, and concentrations of base cations:  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^{+}$  were expressed as  $\text{mg kg}^{-1}$ .

#### **7.2.5            Data Analysis**

Data were analysed by one-way ANOVA with a Tukey HSD *post-hoc* test, and Box-Cox transformations undertaken where necessary to obtain normality.

### **7.3               Results**

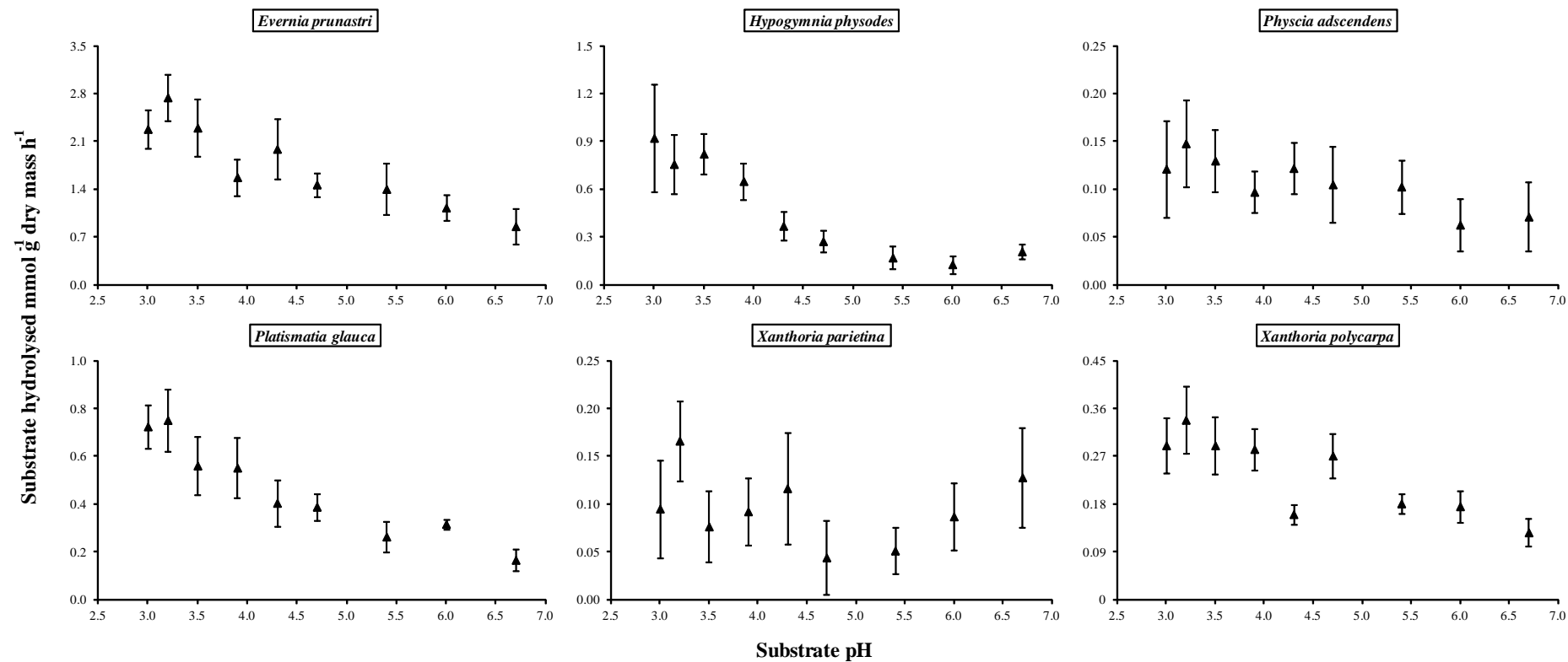
#### **7.3.1            Phosphomonoesterase Activity**

PME activity was readily measureable in all species with marked interspecific variation being observed. The apparent pH optima for PME activity was 3.2 for five of the six species studied. The exception was *Hypogymnia physodes*, which showed the highest activity at pH 3.0 (Figure 7.2). For most species PME activity declined progressively as pH increased, although the distinctiveness of the putative optimum varied from strong (e.g. *Platismatia glauca*) to weak (*Phycia adscendens*). In the case of *Xanthoria parietina*, however, PME activity began to rise again following an initial fall from pH

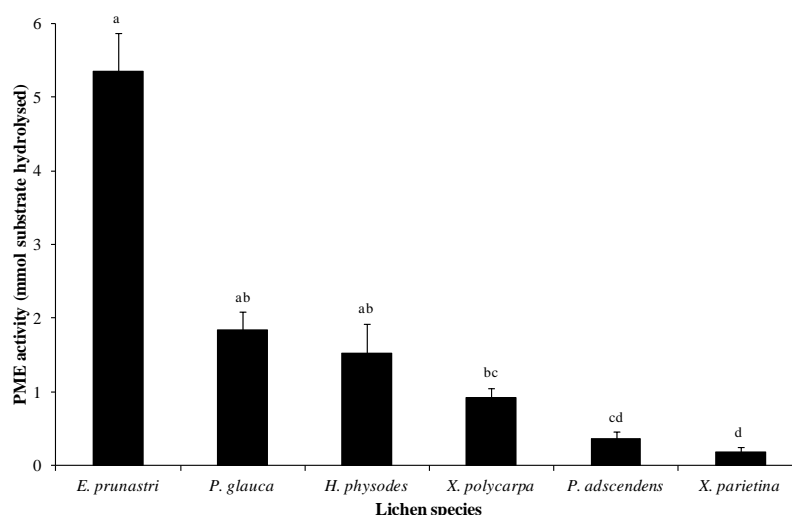
3.2 – 4.7. Subsequently, assays were performed at the two observed optimum values of 3.2 and 6.7 for *X. parietina*.

PME activity in *Xanthoria parietina* measured at pH 3.2 was higher ( $0.187 \text{ mM g}^{-1} \text{ h}^{-1} \pm 0.065$ ) than that at pH 6.7 ( $0.155 \text{ mM g}^{-1} \text{ h}^{-1} \pm 0.065$ ) but, the difference was not significant when the square root transformed data was analysed (one-way ANOVA;  $n = 10$ ;  $df = 1$ ;  $P = 0.755$ ). Activity in *Evernia prunastri* did not differ significantly between the two collection sites [ $5.34 \pm 0.52$  and  $4.36 \pm 0.69 \text{ mmols g}^{-1} \text{ dry mass h}^{-1}$  in the Banchory and Upper Derwent collections respectively (one-way ANOVA;  $n = 10$ ;  $df = 1$ ;  $P = 0.268$ )].

PME activity was significantly greater in the nitrophobic species *Evernia prunastri*, *Platismatia glauca* and *Hypogymnia physodes* compared to the nitrophilic *Physcia adscendens* and *Xanthoria parietina* (Figure 7.3). All of the nitrophobic species possessed mean PME rates  $> 1.5 \text{ mM g}^{-1} \text{ h}^{-1}$ , whereas mean rates for the three nitrophilic species were below  $1.0 \text{ mM g}^{-1} \text{ h}^{-1}$ . Although PME activity in *X. polycarpa* was lower than that in the three nitrophobes, it was not significantly different from that in either *Platismatia glauca* or *Hypogymnia. physodes* (Figure 7.3).



**Figure 7.2** Relationship between PME activity and assay medium pH in six macrolichen species collected from three UK sites (see Table 7.1 for details). Plotted points are mean values  $\pm 1$  standard error of the mean ( $n = 6$ ).



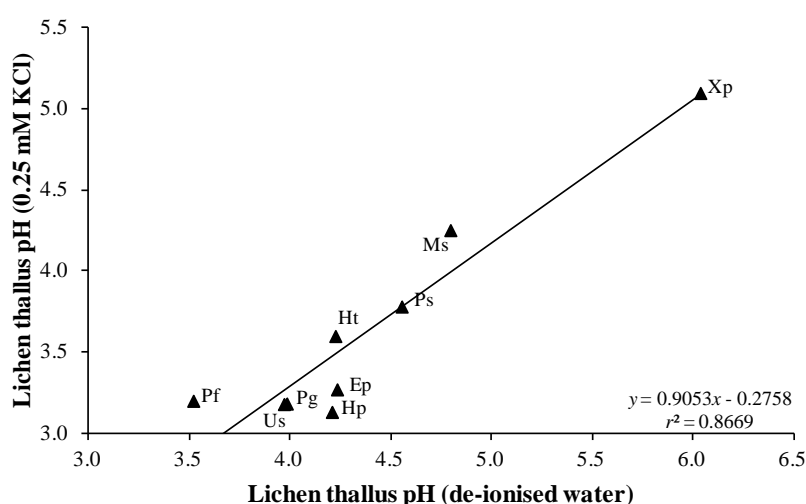
**Figure 7.3** Comparison of phosphomonoesterase (PME) activity among six epiphytic lichens: *Evernia prunastri*, *Hypogymnia physodes* and *Platismatia glauca* (nitrophobic species), *Physcia adscendens*, *Xanthoria parietina* and *X. polycarpa* (nitrophilic species). PME activity was measured at the pH optimum for each species (See Figure 7.2). Error bars represent standard error of the mean ( $n = 10$ ) and columns with the same letter are not significantly different at the  $P = 0.05$  level (one-way ANOVA with Tukey HSD).

### 7.3.2 Effect of Wetting Agent and Equilibration Time on Thallus pH Measurement

The pH values recorded using de-ionised water and 0.25 mM KCl as wetting agents were strongly correlated (Figure 7.4). However, KCl consistently produced more acidic values (Figure 7.4). The six species generally regarded as acidophytes (and which were identified as nitrophobic in Chapter 5) had the most acidic thallus pH values, and were considerably more acidic than *Xanthoria parietina*. The two lichens previously considered as intermediate (Wolseley *et al.*, 2005<sup>b</sup>) included *Parmelia sulcata*, which was identified as a significant nitrophobe in the indicator species analysis (Tables 4.8 and 4.9), and *Melanelixia subaurifera*. The mean thallus pH of these two species was

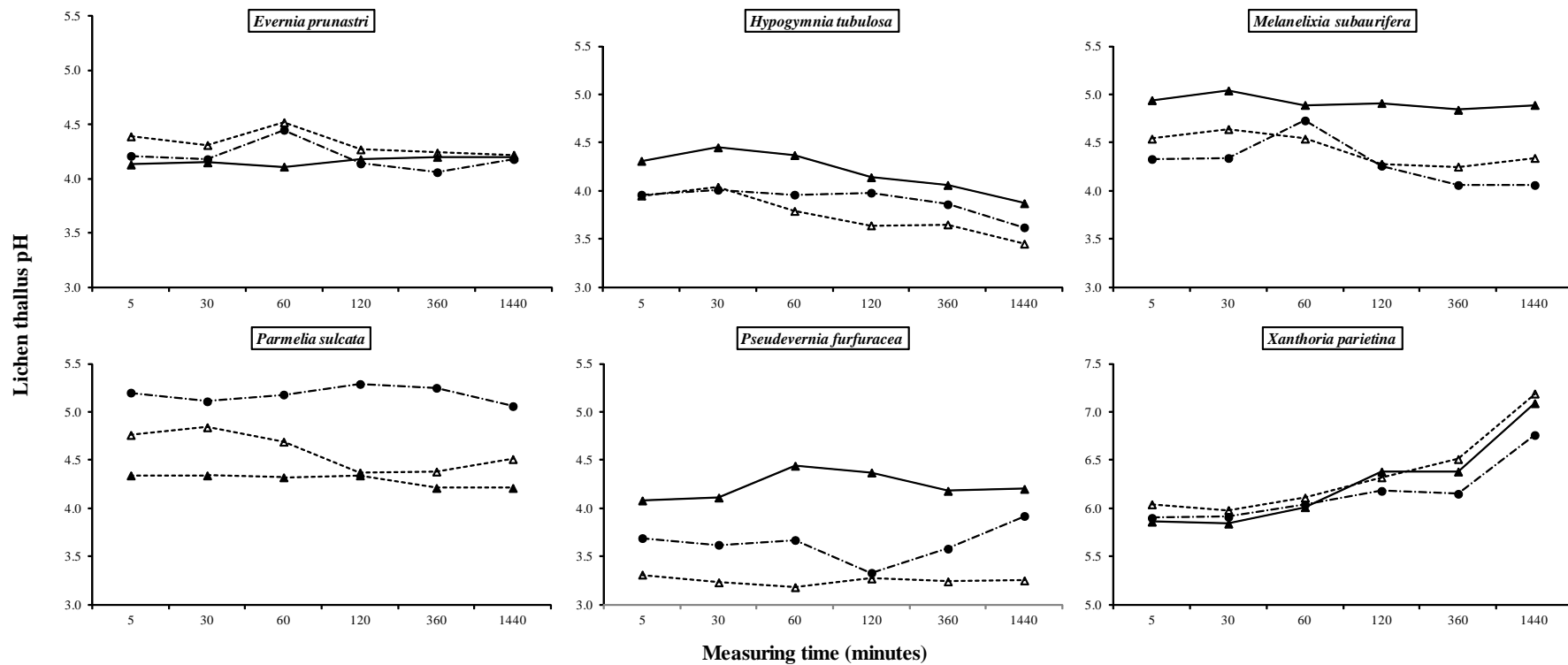
also more acidic than that of *Xanthoria parietina*, but less acid than the established acidophytes/nitrophobes (Figure 7.4).

Measured pH values remained relatively stable over the 24 hour period for all species except those of *Xanthoria parietina*, in which there was a marked increase from the three hour recording point (Figure 7.5). This indicated that thallus pH measurements could effectively be taken almost immediately (within five minutes, as per the limits of the experiment) upon wetting the thallus.



**Figure 7.4** Comparison of lichen thallus surface pH values recorded after 30 minutes in either de-ionised water or 0.25 mM KCl as wetting agents. *Evernia prunastri* (Ep), *Hypogymnia physodes* (Hp), *H. tubulosa* (Ht), *Melanelixia subaurifera* (Ms), *Parmelia sulcata* (Ps), *Platismatia glauca* (Pg), *Pseudevernia furfuracea* (Pf), *Usnea* sp. (Us) and *Xanthoria parietina* (Xp). Mean values (n = 3) are plotted.

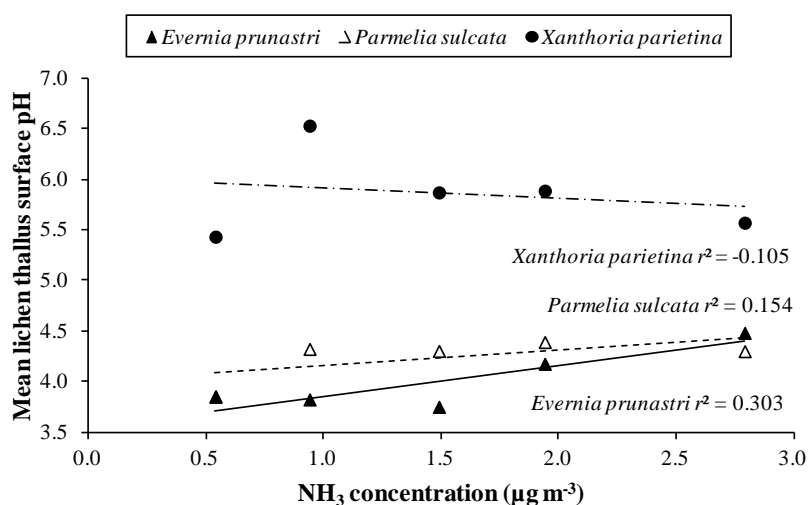




**Figure 7.5** Effect of incubation time on thallus surface pH measurements in six lichen species wetted with de-ionised water. Each line shows progress of pH values in one replicate.

### 7.3.3 Between Site Comparison of Lichen Thalli pH

The mean thallus surface pH in *Xanthoria parietina* (pH 6.24) consistently and significantly higher than that of both *Evernia prunastri* (pH 4.06) and *Parmelia sulcata* (pH 4.32). There was no significant difference between values for the latter two species (Table 7.3), but *E. prunastri* was typically more acidic than *P. sulcata* across the sites (Figure 7.6). There were no strong relationships between site  $[\text{NH}_3]$  and thallus surface pH for any of the three lichen species (Figure 7.6), but the weak positive relationship for *E. prunastri* was significant and reflected the thallus pH increase seen for this species at the most polluted sites (Figure 7.6).



**Figure 7.6** Regression analysis of the relationship between atmospheric  $[\text{NH}_3]$  and the mean lichen thallus surface pH of the three species *Evernia prunastri* (solid line), *Parmelia sulcata* (dotted line) and *Xanthoria parietina* (dot-dash line) at five UK sites with atmospheric  $[\text{NH}_3]$  values ranging from 0.54 to  $2.79 \mu\text{g m}^{-3}$ .

**Table 7.3** The results of a one-way ANOVA to compare the lichen thallus surface pH of three species collected from five UK sites.

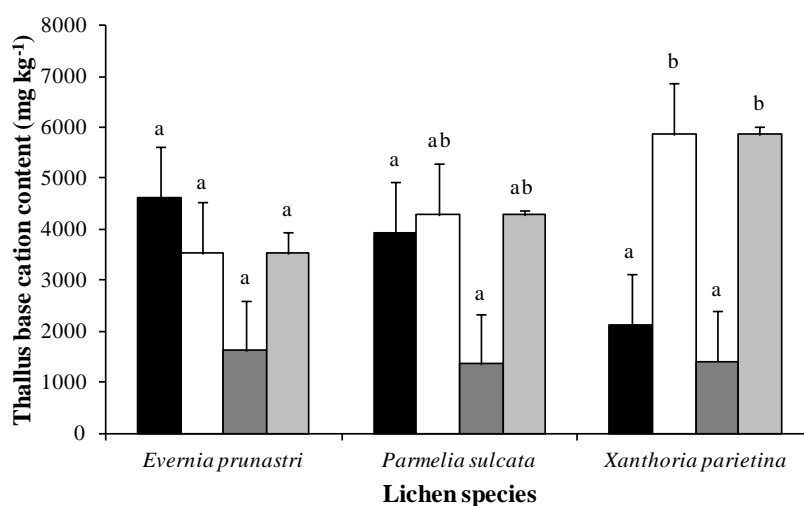
Source	DF	SS	MS	F	P
Lichen thallus pH	2	70.9902	35.4951	159.36	< 0.001
Error	72	16.04	0.2227		
Total	74	87.0269			

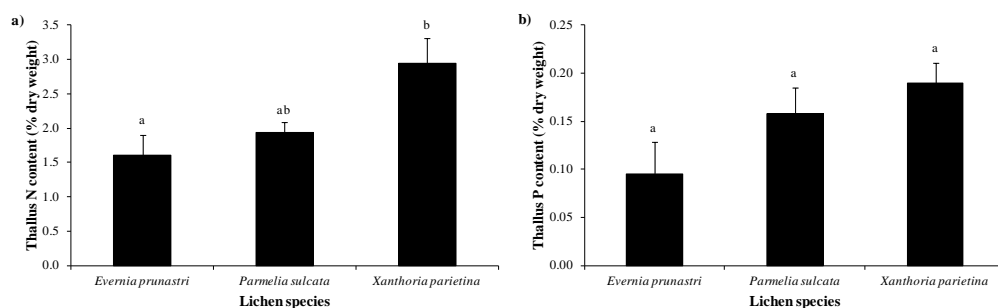
Lichen Thallus pH	n	Mean	SD
<i>Evernia prunastri</i>	25	4.062	0.3409
<i>Parmelia sulcata</i>	25	4.3232	0.3741
<i>Xanthoria parietina</i>	25	6.244	0.6419
Pooled SD			1.0845

#### 7.3.4 Elemental Analysis of Lichen Thalli

No significant difference in thallus concentrations of  $\text{Ca}^{2+}$  (one-way ANOVA;  $n = 4$ ;  $\text{df} = 2$ ;  $P = 0.236$ ) or  $\text{Mg}^{2+}$  (one-way ANOVA;  $n = 4$ ;  $\text{df} = 2$ ;  $P = 0.314$ ) were found between the three species studied. However, *Evernia prunastri* had significantly lower concentrations of  $\text{K}^+$  and  $\text{Na}^+$  (one-way ANOVA;  $n = 4$ ;  $\text{df} = 2$ ;  $P < 0.05$ ) than *Xanthoria parietina* (Figure 7.7). *X. parietina* was also found to contain a significantly (one-way ANOVA;  $n = 4$ ;  $\text{df} = 2$ ;  $P = 0.024$ ) higher %N in the thallus than *E. prunastri* (Figure 7.8). Although thallus %P followed the same trend across the three species, no significant difference was found between *E. prunastri* and *X. parietina* (one-way ANOVA;  $n = 4$ ;  $\text{df} = 2$ ;  $P = 0.095$ ).



**Figure 7.7** Concentrations of the four base cations  $\text{Ca}^{2+}$  (black),  $\text{K}^{+}$  (light grey),  $\text{Mg}^{2+}$  (white) and  $\text{Na}^{+}$  (dark grey) in the thalli of three macrolichen species. Mean values ( $n = 3$ ) are plotted with error bars representing standard error of the mean. Similar lettering between each species indicates non-significant differences for each base cation at the  $P = 0.05$  level based upon a one-way ANOVA analysis with Tukey HSD *post hoc* test.



**Figure 7.8** Total N (a) and P (b) in three lichen species collected from four UK sites (see Table 7.4 for details). Error bars represent standard error of the mean. Different lettering indicates significant differences between species for each base cation at the  $P = 0.05$  level based upon one-way ANOVA analysis with Tukey HSD *post hoc* test.

## 7.4 Discussion

### 7.4.1 Phosphomonoesterase Activity

PME activity was readily measurable in all of the six lichen species examined in this study. In most species there was a clear pH dependence with activity at the optimum pH being greater than at the most sub-optimal value by factors of between 2.4 (*Phycia adscendens*) and 7.2 (*Hypogymnia physodes*) (Figure 7.2). The pH optima was similar in all but two of the species, i.e. *H. physodes* and *Xanthoria parietina*. The pH optima in *H. physodes* was the most acidic, albeit only slightly more so than for four of the other lichens. A distinct pH optimum was less apparent in *X. parietina*, which had the lowest PME activity. The trend for increasing activity at higher pH values suggests the possibility that a true optimum might exceed pH values > 6.7.

There were marked inter-specific differences in PME capacity among the six lichens at the pH optima. There was a trend for higher PME activity in the three nitrophobic species, with *Evernia prunastri* possessing the highest rate of PME activity in this study. Hogan *et al.* (2010<sup>a</sup>) reported that published values of PME activity associated with plant roots and fungal mycelia ranged from • 1 to 7.5 mmol of substrate hydrolysed per g (dry mass) per h, but that the majority of published values are < 1 mmol. Maximum values from Hogan *et al.* for a lichen was 4 mmol g<sup>-1</sup> (dry mass) h<sup>-1</sup>. Accordingly, the rate of PME activity in *E. prunastri* of 5.34 mmol g<sup>-1</sup> (dry mass) h<sup>-1</sup> is remarkably high; and is the highest value yet recorded for a lichen, and one of the highest values in the literature for a plant/fungal system. Crittenden *et al.* (in prep.) examined PME activity in lichens growing in the vicinity of a penguin rookery at Cape

Hallett on the Ross Sea and reported low activity in the nitrophilic *Xanthomendosa borealis* compared to the nitrophobic species *Usnea sphacelata* and *Umbilicaria decussata*. The greater PME activity of the nitrophobic species might be an adaptation to nutrient poor environments where efficient scavenging of P from organic sources is likely to be advantageous. These data in the present study, together with those of Crittenden *et al.* (in prep) provide the first evidence of a physiological difference between nitrophobic and nitrophilic lichens.

#### **7.4.2 Lichen Thallus pH**

The preliminary investigation into the method used to measure lichen thallus surface pH showed that measurements made after 5 mins contact provided reliable measurements of thallus pH. Both KCl and de-ionised water were equally reliable as wetting agents, with KCl producing more acidic results. Measuring time did not affect the results markedly for any of the lichens measured except for *Xanthoria parietina*, which showed a sharp rise in pH at the 24 hour measuring time. The preliminary work also highlighted potential differences in thallus pH linked to the indicator classes of the lichens measured (Figure 7.4), which very broadly reflected the pH optima of the same lichen species examined in the PME activity study. The observed differences in thallus pH were confirmed in the detailed analysis of the three species *E. prunastri*, *Parmelia sulcata* and *X. parietina*, where the nitrophilic *X. parietina* had a significantly higher mean thallus pH across the five sample sites (Table 7.3 and Figure 7.6).

While recent work questions whether phorophyte type plays a greater role than bark pH (Spier *et al.*, 2010), evidence for the effect of pH is widespread. Spore germination under laboratory conditions is dependent on medium composition, including pH (Yamamoto *et al.*, 1998), and studies on the terricolous lichen *Cladonia vulcani* indicated that pH might be important for thallus development (Yoshimura *et al.*, 1987). Furthermore, growth of the photobiont *Trebouxia irregularis* can be significantly reduced by medium pH as well (Ba•kor *et al.*, 1998). In addition to the possible effect on lichen establishment, the findings of the current study provide tentative evidence of the importance of lichen thallus pH to physiological processes.

Inter-site variation in  $[\text{NH}_3]$  and thallus pH for *E. prunastri*, *P. sulcata* and *X. parietina* did not show any strong relationships, but a more marked relationship existed for the nitrophobic *E. prunastri* compared to the nitrophilic *X. parietina* (Figure 7.6). However, the range of  $[\text{NH}_3]$  values included in this comparison was small ( $0.49\text{--}2.79\ \mu\text{g m}^{-3}$ ), a result of the necessity to collect all three species from each sampling site, which limited the number of suitable sites available. It is conceivable that the range of  $[\text{NH}_3]$  values was too small to identify significant changes in thallus pH in the nitrophobic species, even though a trend was identified.

#### **7.4.3 Lichen Thallus Chemistry**

A significantly higher thallus N content was observed in *X. parietina* compared to *E. prunastri*, but not between *P. sulcata* and these two species. None of the thallus P content analyses showed any significant results, however the trend of

increasing thallus content from *E. prunastri* > *P. sulcata* > *X. parietina* found in the N analysis was replicated in the P analysis.

*Xanthoria parietina* has been shown to have a very high tolerance to N deposition, tolerating irrigation of up to 0.17 M NH<sub>4</sub>Cl (1000 kg ha<sup>-1</sup> y<sup>-1</sup>) without suffering damage to either photobiont or mycobiont (Gaio-Oliveira *et al.*, 2004). One hypothesis for nitrophilic species persistence in N-enriched environments is that they possess better coping mechanisms rather than inhibit excessive N uptake. This is supported by the finding by Gaio-Oliveira *et al.* (2001) that *X. parietina* possesses a greater total thallus [N] than *E. prunastri*. Furthermore, it has been suggested by Brown *et al.* (1994) that *X. parietina* may favour habitats with a regular nutrient supply, and as Silberstein *et al.* (1996) comments, this may benefit the lichen as its resistance to sulphur-based pollution is believed to be due to induced arginine synthesis and a high K content. No conclusion regarding the relationship between arginine and S resistance can be made from this current study. However, the significantly higher K<sup>+</sup> concentrations in *X. parietina* found in this study (Figure 7.7) appear to support this statement, and field observations across the regional sites showed that *X. parietina* was abundant both at sites with high [N] and where previous SO<sub>2</sub> perturbation had occurred.

The relationship between elevated atmospheric [NH<sub>3</sub>], thallus N accumulation and removal through arginine synthesis is well recorded. Increases in total tissue N and arginine have been found to be closely linked to [NH<sub>3</sub>] and N deposition in a study of three Bryophyta species (Pitcairn *et al.*, 2003), and



research by Dahlman *et al.* (2003) showed that arginine concentrations in the nitrophobic *Hypogymnia physodes* and *Platismatia glauca* increased by ca. 60-fold following intensive  $\text{NH}_4\text{NO}_3$  fertilisation. The increased production of arginine has also been described as being an early response to P limitation (Rabe & Lovatt, 1986). This raises questions on the relative importance of P limitation and thallus arginine increases to lichen health in areas of N-enrichment, and whether thallus arginine levels can be used as a biochemical method to indicate pollution levels in lichens.

## Chapter 8                      General Discussion

This study critically evaluated macro- and microlichens using quantitative analysis for concentrations of  $\text{NH}_3$  gas in air for the first time. The work resulted in the production of a comprehensive list of indicator species for  $[\text{NH}_3]$  that could be used in a bio-monitoring scheme. A total of 65 species (58 lichens and 7 bryophytes) were identified as possible indicators using Indicator Species Analysis (ISA) (Section 4.8.1), and these were supported in the ordination analysis (Section 4.7.2). Of the indicators identified, 66% were species previously described as putative indicators in earlier literature (van Herk, 1999; Wolseley, 2005; Wolseley *et al.*, 2005<sup>a</sup>; Wolseley *et al.*, 2009). A number of previously established indicator species, such as *Diploicia canescens*, *Hypocenomyce scalaris*, *Phaeophyscia nigricans* and *Physconia distorta* were not identified as such in the current analysis (Section 4.9.7). This was because they were either not recorded during the survey or excluded from analysis because they were recorded at too few sites ( $n < 3$ ). Consequently, the nitrophobic and nitrophilic indicator species identified in this study should be accepted as being complimentary to, rather than a replacement of, established lists. The majority of newly identified indicators were microlichens, although some new macrolichens (e.g. *Sphaerophorus globosus*) were also identified. The most marked observation was that all of the fruiticose lichens identified as indicators were nitrophobic and none nitrophilic.

Two lichen species in the ISA were seen to contradict established knowledge of their indicator status, these were *Chaenotheca ferruginea* and *Lepraria*

*incana*. Both species were identified as nitrophilic in the tree trunk analysis, but previously described as acidophytic (nitrophobic) by both van Herk (1999) and Wolseley *et al.* (2009). There are two possible explanations for this apparent contradiction. First, the strongest relationships for these two species may have been with a variable other than N, e.g. bark pH. Both species were traditionally listed as acidophytic (van Herk, 1999), and the trunk bark of older trees, where the presence of both species was most evident, is likely to be more acidic due to leaching effects (Legrand *et al.*, 1996). It was not possible to accurately evaluate this hypothesis as measurements of tree age were not incorporated into this study. Second, tree age might be linked to species distributions in another way, as both *C. ferruginea* and *L. incana* are known to prefer dry bark recesses (Smith *et al.*, 2009) that are more prominent on the older, more rugose bark of mature trees. Coincidentally, the older trees (as determined by size) were located in sites where the  $[\text{NH}_3]$  levels were above the  $1 \text{ mg m}^{-3}$  threshold set in the ISA to determine indicator species (personal observation). A final item of note is that *C. ferruginea* was only recorded at four sites (Appendix F), which may have influenced the statistical analysis.

There was sufficient overlap in the presence of indicator species on both trunks and branches of the surveyed tree species to enable the production of a simple indicator list for both nitrophobes and nitrophiles that could be used on both substrata. A subjective selection method was undertaken to establish a shortlist of easily identifiable indicators (Table 4.10) from the lists produced in this study that were well distributed across the UK and were less likely to be influenced by climate. Species were also chosen from those found growing on

the trunks and branches of both *Betula* and *Quercus* trees. All the analysis involving the use of indicator species was undertaken using this one shortlist, and the species comprising it correlated well with [NH<sub>3</sub>] values. The possible benefit of using a combination of shortlisted indicator species different from that used in the evaluation of the scoring system (Table 4.10) was not addressed due to time constraints. It is possible that stronger, more robust results might be obtained using a different combination of species from the main indicator lists produced in the ISA (Tables 4.8 & 4.9), and this is something that might warrant further investigation.

A new bio-monitoring scheme based on Wolseley *et al*'s. (2009) Lichen Acidophyte Nitrophyte (L<sub>AN</sub>) method, using a limited number of indicators, was proven to be an effective means of evaluating NH<sub>3</sub> pollution levels. The trialling of different scoring methods, including three approaches weighted for species richness (SR), showed that an un-weighted scoring system (i.e. the NUFL<sub>AN</sub>), produced the most reliable results based on the survey data obtained. However, its effectiveness is ultimately reliant upon the shortlist of indicator species in terms of their efficacy as [NH<sub>3</sub>] indicators, ease of identification, widespread UK distribution and their relative sensitivity to other environmental variables.

Problems relating to lichen identification by non-specialists mean that most species used in the indicator lists are still likely to be macrolichens, as in the case of the study by Wolseley *et al.* (2009). This is not expected to be problematic given the strong relationships observed between [NH<sub>3</sub>] values and

both measured and modelled  $\text{NUFL}_{\text{AN}}$  scores obtained from the lichens selected for the shortlist of indicators in this study (Sections 5.3.2.2 and 5.3.3). Despite the limited use of microlichens identified as indicators in the analysis, the knowledge of their relevance can provide a more detailed overview of site condition by individuals with more advanced identification skills beyond the use of the bio-monitoring scheme. This is likely to be of particular importance for branch surveys where the relative SR of microlichens can be greater than that of macrolichens. Conversely, the matter regarding species distributions across the UK is important given the historical perturbation that may have occurred across much of the country as a result of past pollution and/or land management practices. Consequently, the bio-monitoring scheme is likely to be most reliable in natural or semi-natural habitats with minimal historical disturbance.

Another issue in using the  $\text{NUFL}_{\text{AN}}$  scoring method as part of the bio-monitoring scheme is the disproportionate effect of a single thallus of a conflicting indicator species on the typical community composition where a basic single quadrat per aspect approach is used (Section 5.4.1). The utilisation of a ladder transect, as used in the regional scale survey (Section 2.9) and described by Wolseley *et al.* (2009), on each aspect is strongly recommended to offset this. Recording the presence of indicator species in the five quadrats of the ladder transect, as opposed to the simple presence of indicators on the trunk aspect, will improve the resolution of the survey and reduce the weighting influence of a single thallus of one indicator type with restricted occurrence amongst a community of the opposing indicator species. Two

further recommendations for the use of the scheme are to restrict surveys to certain tree species within a limited age range. This is supported by the finding that  $\text{NUFL}_{\text{AN}}$  scores had considerably stronger correlation with surveys undertaken on *Betula* compared with those on *Quercus*, and might be influenced by tree age. Surveying within these constraints at the site level would eliminate any distortion in the data as a result of these factors, although this is probably not practical on a nationwide scale. Furthermore, in an attempt to eliminate variation caused by alkaline dust deposition on surveyed trees, sites on calcareous soils were excluded from the study. Consequently, the robustness of the method is not tested on such sites, and further research is required in this area to determine what influence these soil conditions might have, and whether the method is reliably transferrable to such locations.

The regression models incorporating  $[\text{NH}_3]$ ,  $[\text{NO}_2]$  and bark pH as the key variables of the shortlisted indicator species explained a high proportion of variance in the observed  $\text{NUFL}_{\text{AN}}$  scores on trunks (81%) and branches (87%). The decreased ratio of  $[\text{NH}_3]$  to both  $[\text{NO}_2]$  and pH in the equation for branches (Eq. 5.3) compared to trunks (Eq. 5.2) provides evidence that the relative effects of  $\text{NO}_2$  and bark pH are greater on the branches. Despite this, relationships between observed  $\text{NUFL}_{\text{AN}}$  scores and modelled calculations based on the compromise model  $\text{NUFL}_{\text{AN}} = 5 \cdot [\text{NH}_3] + 2 \cdot [\text{NO}_2] + \text{pH}$  were very strong for both trunks ( $r^2 = 0.816$ ) and branches ( $r^2 = 0.811$ ). This showed that a single model could be used as the foundation to estimating  $\text{NH}_3$  values for both substrata, regardless of the potentially confounding effects of  $\text{NO}_2$  and pH. A similar study was previously undertaken by Wolseley *et al.* (2005<sup>a</sup>),

which reported that the relative effects of pH and  $\text{NH}_3$  were biased towards the former by a factor of four. The switch in dominant variable from pH to  $[\text{NH}_3]$  shown in this PhD study provides further support to the reliability of the indicator species used in determining  $\text{NUFL}_{\text{AN}}$  scores on the basis of N pollution. The strong relationship observed in this study also indicated that the model is a robust method of assessing  $[\text{NH}_3]$  across the range of 0.03 to  $7.56 \mu\text{g m}^{-3}$ , as recorded at the 28 surveyed sites. This confirmed the models suitability to evaluate conditions below the United Nations Economics Commission for Europe (UNECE) long-term  $[\text{NH}_3]$  critical level (CLE) for sensitive habitats of  $1 \text{ mg m}^{-3}$ , and well beyond the standard CLE of  $3 \mu\text{g m}^{-3}$ , further validating its use across the UK as a whole.

The inclusion of  $[\text{NO}_2]$  and bark pH into the model compromised the ability to estimate site  $[\text{NH}_3]$  values directly from measured  $\text{NUFL}_{\text{AN}}$  scores. This problem can be alleviated by obtaining  $[\text{NO}_2]$  values from established models such as the Fine Resolution Atmospheric Multi-pollutant Exchange (FRAME) and Simple Calculation of Atmospheric Impact Limits (SCAIL) (Apis, 2012) and by making bark pH measurements. However, an alternative method would be to combine  $[\text{NH}_3]$  and  $[\text{NO}_2]$  values together into an overall air N index value that is calibrated to the UNECE CLE values of both N forms. If such an approach proves successful it would mean that it might be possible to use the bio-monitoring scheme beyond the remit of sensitive rural habitats and even include more urban locations where  $[\text{NO}_2]$  is the dominant N source.

The evaluation of NUFL<sub>AN</sub> scores against atmospheric chemistry and climate variables showed that other relationships existed, some of which were incorporated into a regression model. Most notable of these were [NO<sub>2</sub>], bark pH and climate (as defined by RD > 1mm). For simplicity, only the two former variables were added to the model as confounding factors. Climate was excluded on the basis that the cumulative amount of variation explained by adding this variable to the regression equation was very low (Section 5.3.3). An attempt was made to exclude indicator species that were potentially influenced by climate, such as *Hypotrachyna revoluta* from the short list of indicators, and hence, the model. Given the observed polarisation of climate and atmospheric chemistry data in the ordination analyses (Figures 4.6-4.9) it is imperative that consideration is given to the effect of climate on species in the main indicator lists produced in this study (Tables 4.9-4.10) if species other than those selected for the shortlist of species (Table 4.10) are used.

The finding that bark pH had an effect on lichen community composition independent of [NH<sub>3</sub>], as defined by both the relationship with NUFL<sub>AN</sub> scores and the additive effect during construction of the regression model supports previous reports of a link between pH and lichen communities. These include van Herk's (2001) conclusion that epiphytic community change was a result of NH<sub>3</sub>-driven increases in substratum pH and both Gilbert (1976) and Loppi *et al.* (1997) showing that pH changes due to the hypertrophicating effects of dust impregnation had a similar effect on community composition. The greater influence of pH on the branches alludes to the possibility that its effect is important in early thallus development and colonisation. Support for this



comes from Yoshimura *et al.* (1987), who identified in a study on *Cladonia vulcani* that the equal growth rates of the different bionts coincided with the natural pH of the habitat where the lichen was typically found. Furthermore, Yamamoto *et al.* (1998) showed that spore germination of lichens was influenced by pH.

The link between  $\text{NUFL}_{\text{AN}}$  scores and  $[\text{NO}_2]$  was not wholly unexpected as previous studies have shown a positive connection between  $\text{NO}_2$  and nitrophilic/nitrophytic lichens (Gadsdon *et al.*, 2010; van Dobben & ter Braak, 1998). The positive relationship between  $[\text{NO}_2]$  and branch bark pH (Table 5.1) was an unexpected discovery, and no previous evidence of such a positive relationship between the two variables is known. Investigations have previously been undertaken by both Marmor & Randlane (2007) and van Dobben & ter Braak (1998) where measurements of  $\text{NO}_2$  and pH were made, but no relationship between the two reported. Furthermore, any conceivable relationship between  $[\text{NO}_2]$  and bark pH might be expected to be negative, given that wet deposition of the the oxidised N form would logically be as  $\text{HNO}_3$  *via*  $\text{NO}_2$  oxidation into  $\text{NO}_3^-$ . However, the finding at Whim Moss that surface pH increased with  $\text{NO}_3^-$  application, potentially as a result of biological processes, suggests that dry deposition of  $\text{NO}_2$  onto surfaces might cause a surface pH increase following oxidation of  $\text{NO}_2$  to  $\text{NO}_3^-$ . Further studies need to be undertaken regarding this issue to discern whether a valid relationship between  $[\text{NO}_2]$  and bark pH exists.

Several other variables were shown to correlate with NUFL<sub>AN</sub> scores, and consequently the distributions of the indicator species used. These were typically excluded on the basis of their co-correlation with other variables (Section 3.3.5), such as S compounds, which co-correlated positively with N compounds (i.e. [SO<sub>2</sub>] with [NO<sub>2</sub>] and [SO<sub>4</sub><sup>2-</sup>] with [NH<sub>4</sub><sup>+</sup>] and [NO<sub>3</sub><sup>-</sup>]), but negatively with NUFL<sub>AN</sub> scores. In some instances exclusion was not as clear cut, the most notable example being the relationship between NUFL<sub>AN</sub> scores and aerosol [NH<sub>4</sub><sup>+</sup>] (Figure 5.4). Both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> are well documented as major N sources for plants (Jauhiainen *et al.*, 1998; Leith *et al.*, 1999), and have been identified as potentially detrimental to oligotrophic habitats and the species found within them (Gordon *et al.*, 2001; Fremstad *et al.* 2005; Leith *et al.*, 1999; Tomassen *et al.*, 2004). This was supported by the negative relationship between NUFL<sub>AN</sub> scores and aerosol [NH<sub>4</sub><sup>+</sup>]. However, a strong relationship between NUFL<sub>AN</sub> scores and aerosol [NH<sub>4</sub><sup>+</sup>] is perhaps inevitable given that NH<sub>3</sub> is the main source of reduced N, and correlation analysis of [NH<sub>3</sub>] and [NH<sub>4</sub><sup>+</sup>] showed that this was the case (Table 3.5). Finally, although a relationship was shown between aerosol [NH<sub>4</sub><sup>+</sup>] and NUFL<sub>AN</sub>, no significant relationship was detected between wet deposited NH<sub>4</sub><sup>+</sup> and NUFL<sub>AN</sub> scores (Figure 5.4). As NH<sub>4</sub><sup>+</sup> is more typically measured as a deposition value rather than concentration in aerosol, it would be misleading to include it as confounding variable. Despite this, the relative importance of both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on lichen communities remains an issue warranting further investigation.

The study undertaken at Whim Moss highlighted how the community composition varied in the NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> treatment plots, with nitrophobic

species such as *Evernia prunastri* and *Hypogymnia* spp. colonising the more acidic substrata and the nitrophilic *Xanthoria parietina* and *Physcia tenella* the less acidic substrata, regardless of N application (Figure 6.6). One explanation for this is that, although N-enrichment and N-form are important in community composition, differences in substratum pH play an instrumental role in lichen colonisation and development. This would support the relative importance of pH in early lichen colonisation as seen in the regional-scale survey results where  $\text{NUFL}_{\text{AN}}$  scores were lower, or more negative, on the less acidic *Quercus* compared to *Betula* trees at sites where both were surveyed.

Finally, the importance of PK must be considered, especially when N is sufficiently available, or present at particularly high levels. This became evident in the findings at Whim Moss where lichen colonisation and growth was exclusively restricted to the N treatment plots which also had PK applied. One reason this pattern emerged is that PK was seen to be the limiting factor to successful lichen colonisation in the N treated wet deposition plots.

PME activity was shown to be readily measurable in a range of epiphytic lichens and an acid pH optima of 3.0-3.2 was recorded in all instances except for *Xanthoria parietina*. This species had a relatively flat response over the pH range assessed, although the results obtained do not preclude the possibility of an alkalai pH optimum for it. Therefore further investigations are required to clarify this issue.

A physiological trend was identified across the indicator species whereby the three nitrophobic species *Evernia prunastri*, *Hypogymnia physodes* and *Platismatia glauca* had higher rates of PME activity than the nitrophilic species *Physcia adscendens*, *Xanthoria parietina* and *X. polycarpa* (Figure 7.3). The rate of PME activity for *E. prunastri* was significantly higher than the other five species (Figure 7.3) and even greater than *Cladonia portentosa*, as reported by Hogan *et al.* (2010<sup>a</sup>). Furthermore, to current knowledge it is one of the highest rates known to be recorded in biological systems, including those of arbuscular and ectomycorrhizal fungi (Joner *et al.*, 2000; McElhinney & Mitchell, 1993). *E. prunastri* was also the only one of the six species with a growth habit more typical of fruticose lichens (Smith *et al.*, 2009).

The significantly more acidic lichen thallus surface pH of *E. prunastri* compared to *X. parietina* in specimens collected from the regional-scale survey sites may help explain PME activity differences. It is possible that the optimum pH of PME activity in lichens is an adaptation to the environment of the thallus. If PME activity is linked to thallus surface pH, which itself is a physiological difference between nitrophobic and nitrophilic species, then thallus pH measurements might prove a quick initial means of identifying potential indicator species. Further detailed research investigating potential relationships between thallus surface pH and PME activity of indicator species is required to confirm this.

The work undertaken in this study has produced quantitative evidence for the establishment of lichen indicator species that can be used to detect N pollution.

Investigations into the biochemistry of indicators also provided evidence of a physiological difference between nitrophobes and nitrophiles, which supported the validity of their use. Combined with the formulation of a robust scoring system, this has provided a strong underpinning for the creation of a field guide that can be used by government agencies and conservation bodies to identify threats to designated sites by N enrichment. The use of epiphytic lichens has made it particularly useful with regards to the assessment of sensitive habitats where there is a need to detect low levels of  $\text{NH}_3$ , as determined by the UNECE CLE. Furthermore, the finding that the indicator species identified and used in this study reacted in a similar manner to both  $\text{NH}_3$  and  $\text{NO}_2$  implies that use of the bio-monitoring scheme could be expanded beyond the original remit of evaluating reduced N in sensitive habitats in rural areas to those where oxidised N sources are dominant.

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## Appendix A – UK sites evaluated for the regional-scale epiphyte survey

Site Name	Region	Grid Ref	Reason for Omission
Alice Holt	Hampshire	SU 809 379	Monitoring station closed down and relocated to Alice Holt 2.
Alice Holt 2	Hampshire	SU 805 427	Site selected.
Allt a Mharcaidh NNR	Highlands	NH 891 043	High altitude and few trees.
Ariundle	Highlands	NM 835 642	Site dropped due to problems in obtaining permission.
Aston Rowant NNR	Oxfordshire	SU 727 979	Calcareous/limestone pedology and/or geology.
Auchencorth Moss (LHS)	Midlothian	NT 221 562	Trees absent.
Auchincruive	East Ayrshire	NS 379 234	Intensively grazed farmland with few trees.
Banchory	Aberdeenshire	NO 676 985	Site selected and ALPHA sampler monitoring station established.
Barcombe Mills	Sussex	TQ 438 149	Few trees in area.
Bedingfield	Suffolk	TM 173 684	Arable fields with pig and poultry farms and few trees.
Bickerton Hill NNR	Cheshire	SJ 498 527	Insufficient information. No response from landowner/Government body.
Borrowdale	Cumbria	NY 245 160	Insufficient information. No response from landowner/Government body.
Bretton Lakes	West Yorkshire	SE 282 123	Site selected and ALPHA sampler monitoring station established.
Brompton	North Yorkshire	SE 389 988	No suitable trees present.
Brown Moss LNR	Shropshire	SJ 560 396	Monitoring station closed down in 1998 and relocated to Brown Moss 2.
Brown Moss 2 LNR	Shropshire	SJ 563 390	Site selected.
Bure Marshes NNR	Norfolk	TG 334 161	Insufficient information. No response from landowner/Government body.
Bush Estate	Midlothian	NT 245 635	Site selected but survey limited because of permission limitations.
Caenby	Lincolnshire	SK 993 900	Insufficient information. Few trees identified at location.
Caldanagh Bog	Co. Antrim	ID 022 205	Unsuitable tree species.
Cardigan SSSI	Cardiganshire	SN 185 453	Insufficient information. Nearby site (Tycanol Wood) selected.
Cardoun Burn	Dumfries & Galloway	NX 546 658	Insufficient information. No response from landowner/Government body.
Carlisle	Cumbria	NY 468 554	Insufficient information.
Carradale	Argyll & Bute	NR 798 378	Calcareous/limestone pedology and/or geology.
Castle Cary LNR	Somerset	ST 609 319	No response from landowner/Government body.
Castle Enigan	Co. Down	IJ 121 322	Unsuitable tree species.
Chafer Wood	North Yorkshire	SE 899 832	Calcareous/limestone pedology and/or geology with unsuitable tree species.
Close House	Northumberland	NZ 128 657	Unsuitable tree species.
Coalburn	Camarthenshire	SN 816 484	Relocated to Llyn Brianne.
Coleraine	Co. Londonderry	IC 884 211	Insufficient information.
Correl Glen NNR	Co. Fermanagh	IH 075 544	Site selected and nearby monitoring station (Lough Navar) used.
Cymystwyth	Ceredigionshire	SN 771 742	Site selected.
Dennington	Suffolk	TM 276 669	No suitable trees present.
Detling	Kent	TQ 801 597	Insufficient information. No response from landowner/Government body.
Drayton	Warwickshire	SP 162 551	Arable fields with pig and poultry farms with few trees.
Dunalastair Estate	Perthshire	NN 748 600	Site selected and nearby monitoring station (Tummel) used.
Dunwich Heath SSSI	Suffolk	TM 470 680	Coastal heathland.
Dyffryn Mymbyr NNR	Gwynedd	SH 695 573	Monitoring station closed down and relocated to Plas y Brenin.
Easingwold	North Yorkshire	SE 540 675	Unsuitable trees.
East Saltoun	East Lothian	N/K	Insufficient information. No response from landowner/Government body.
Edinburgh St. Leonards	Midlothian	NT 262 731	Urban environment.
Edinburgh-Johnston Terrace	Midlothian	NT 253 734	Urban environment.
Edinburgh-Medical School	Midlothian	NT 263 726	Urban environment.
Ellon Ythan	Aberdeenshire	NJ 945 304	Coastal site with no suitable tree species (conifer plantation).
Eskdalemuir	Dumfries & Galloway	NT 235 030	Upland moorland with conifer plantation.
Fenns Moss 1 NNR	Wreccsam	SJ 498 378	No suitable trees present.
Fenns Moss 2 NNR	Wreccsam	SJ 498 368	No suitable trees present.
Fenns Moss 3 NNR	Wreccsam	SJ 498 378	No suitable trees present.
Five Acres LNR	Cornwall	SW 794 486	No response from landowner/Government body.
Fressingfield	Suffolk	TM 261 759	Farmland with few trees.
Frodsham LNR	Cheshire	SJ 525 795	Downwind of several chemical installations.
Garbutt Wood	North Yorkshire	SE 506 834	Calcareous/limestone pedology and/or geology.
Glen Nant ASSI	Argyll & Bute	NN 013 280	Site selected and ALPHA sampler monitoring station established.
Glenmore Wood	Co. Tyrone	IH 651 609	Site selected and ALPHA sampler monitoring station established.
Glensaugh	Grampian	NO 664 799	Upland moorland with few suitable trees.
Glenshee NNR	Perthshire	NO 121 690	Monitoring station closed down and relocated to Gulabin Lodge.
Glenshee Hotel	Perthshire	NO 111 699	Monitoring station located on roadside.
Globeflower Wood	North Yorkshire	SD 873 667	Calcareous/limestone pedology and/or geology.
Goonhilly	Cornwall	SW 723 214	No suitable trees present.
Grass Wood	North Yorkshire	SD 983 652	Calcareous/limestone pedology and/or geology.
Griffin	N/K	N/K	Insufficient information. No response from landowner/Government body.

Site Name	Region	Grid Ref	Reason for Omission
Grizedale Forest	Cumbria	SD 337 941	Site selected.
Gulabin Lodge NNR	Perthshire	NO 110 701	Unsuitable habitat and monitoring ended Dec 2006.
Halladale	Sutherland	NC 902 488	Site dropped due to problems in obtaining access.
Harwell	Oxfordshire	SU 474 863	Built environment.
High Muffles NNR	North Yorkshire	SE 776 939	Forestry plantation.
Hillsborough	Co. Down	IJ 243 577	Insufficient information.
Hinderclay Fen	Suffolk	TM 025 787	Site selected and ALPHA sampler monitoring station established.
Holme Lacy	Herefordshire	SO 554 357	Monitoring station closed down and relocated to Rosemaund.
Inverpolly B NNR	Sutherland	NC 187 088	Birch woodland located >15 km from monitoring station.
Jenny Hurn	Lincolnshire	SK 816 986	Monitoring station closed down and relocated to Marton.
Knockan	Highlands	NC 187 088	Unsuitable tree species.
Ladybower	Derbyshire	SK 164 892	No response from landowner/Government body.
Lagganlia	Highlands	NH 856 037	Insufficient information.
Lakes	Cumbria	SD 337 941	Monitoring station closed down and relocated to Grizedale Forest.
Little Budworth NNR	Cheshire	SJ 584 658	Insufficient information.
Llydaw NNR	Gwynedd	SH 639 551	Monitoring station closed.
Llyn Brianne	Camarthenshire	SN 816 484	Conifer plantation.
Llyncllys Common SSSI	Shropshire	SJ 274 236	Calcareous/limestone pedology and/or geology.
Loch Awe	Argyll & Bute	NM 966 115	Nearby site being surveyed (see Glen Nant).
London, Bloomsbury	London	N/K	Urban environment.
London, Cromwell Rd	London	N/K	Urban environment.
London, Victoria	London	N/K	Urban environment.
Lough Navar	Co. Fermanagh	IH 065 545	See Correll Glen.
Low Wood	West Yorkshire	SE 056 439	Site dropped due to late response from landowner.
Lullington Heath NNR	East Sussex	TQ 538 016	Calcareous/limestone pedology and/or geology.
Lyulphs Tower	Cumbria	NY 403 202	Few trees in area.
Marton	Lincolnshire	SK 844 818	Monitoring station closed.
Mere Sands Wood LNR	Cheshire	SD 447 157	Site selected.
Midge Hall	Lancashire	SD 508 231	Monitoring station in garden adjacent to cattle-grazed fields.
Moninea Bog ASSI	Co. Fermanagh	IH 301 217	Site selected and ALPHA sampler monitoring station established.
Moor House NNR	Durham	NY 751 334	Upland morrland with few trees.
Much Hoole	Lancashire	SD 473 231	Monitoring station in garden adjacent to cattle-grazed fields.
Myerscough	Lancashire	SD 498 399	Unsuitable tree species on intensely grazed grassland.
Narbeth NNR	Pembrokeshire	SN 146 127	Insufficient information.
North Cliffe Wood	East Riding of Yorkshire	SE 860 374	No response from landowner/Government body.
North Wyke	Devon	SX 659 983	Site selected.
Northallerton	Yorkshire	SE 360 930	Urban environment.
Oldmeldrum	Aberdeenshire	NJ 832 273	Oak woodland located > 1 km from monitoring station.
Orielton NNR	Pembrokeshire	SR 982 947	Monitoring station relocated to Narbeth.
Pen y Garn	Ceredigionshire	SN 798 771	Agricultural land and forestry plantation.
Penallt SSSI	Monmouthshire	SO 523 095	Unsuitable trees.
Pitmedden	Aberdeenshire	NJ 883 278	Monitoring station closed down.
Plas y Brenin	Gwynedd	SH 716 578	Site selected.
Plynlimon	Ceredigionshire	SN 822 841	Unsuitable tree species.
Pointon	Lincolnshire	TF 128 313	Intensive arable farmland with few trees.
Polloch	Invernesshire	N/K	Insufficient information.
Porton Down NNR	Wiltshire	SU 253 365	No response from landowner/Government body.
Priddy SSSI	Somerset	ST 525 526	Monitoring station closed down.
Pwllpeiran	Ceredigionshire	N/K	Insufficient information.
Rannoch Forest SSSI	Perthshire	NN 603 533	Site selected.
Redgrave & Lopham Fen NNR	Suffolk	TM 053 802	Site selected.
Rochester	Kent	N/K	Insufficient information. No response from landowner/Government body.
Rosemaund	Herefordshire	SO 564 476	Insufficient information.
Rothamsted	Hertfordshire	TL 123 129	Site selected.
Ruabon NNR	Wrexham	SJ 225 489	Site selected.
Rum NNR	Highlands	NM 408 992	Trees unsuitable for survey.
Savarnake	Wiltshire	SU 055 888	No response from landowner/Government body.
Sheffield	South Yorkshire	SK 332 870	Relocated to Sheffield 2.
Sheffield 2	South Yorkshire	SK 339 873	Urban environment.
Sherwood	Derbyshire	SK 163 905	Site closed.



Site Name	Region	Grid Ref	Reason for Omission
Shetland	Shetland	HU 454 396	Trees absent.
Sibton	Suffolk	TM 363 722	Insufficient information.
Silsoe	Bedfordshire	TL 088 356	Arable fields with few trees.
Sourhope	Borders	NT 867 218	Upland grassland with conifer plantation.
Stackpole	Pembrokeshire	SR 982 947	Monitoring station closed down and relocated to Narbeth.
Stanford SSSI	Norfolk	TL 859 948	Monitoring station closed down and relocated to Stanford 2.
Stanford 2 SSSI	Norfolk	TL 858 948	No response from landowner/Government body.
Stoke Ferry	Norfolk	TL 700 988	No response from landowner/Government body.
Stoneycliffe Wood	West Yorkshire	SE 274 161	No response from landowner/Government body.
Strath Vaich Dam	Highlands	NH 348 750	Site selected.
Sutton Bonnington	Nottinghamshire	SK 505 268	Site selected.
Swannington Upgate Common SSSI	Norfolk	TG 148181	High work commitment requested from contact.
Sweetenham Meadows LNR	Cheshire	SJ 804 674	Insufficient information. No response from landowner/Government body.
Tadcaster	North Yorkshire	SE 478 442	Insufficient information.
Thetford	Norfolk	TL 944 841	No response from landowner/Government body.
Thursley Common SSSI	Surrey	SU 910 404	No response from landowner/Government body.
Torrisdale Cliff SSSI	Argyll & Bute	NR 798 378	Calcareous/limestone pedology and/or geology.
Tummel Forest	Perthshire	NN 744 611	Site dropped as permission withdrawn.
Twentywellsick Wood	South Yorkshire	SK 326 808	Site selected and ALPHA sampler monitoring station established.
Tycanol Wood	Cardiganshire	SN 090 366	Site selected.
Upper Ballinderry River ASSI	Co. Tyrone	IH 765 787	Site selected and ALPHA sampler monitoring station established.
Victoria/Cromwell Road	London	TQ 266 791	Urban environment.
Wardlow Hay Cop NNR	Derbyshire	SK 177 737	Few trees in area.
Wem Moss NNR	Shropshire	SJ 473 343	Insufficient information.
Westhay Moor LNR	Somerset	ST 455 437	Reedbed, meadow and pastureland. Few trees in area.
Wood of Cree SSSI	Dumfries & Galloway	NX 386 716	Site selected and ALPHA sampler monitoring station established.
Wynbury Moss NNR	Cheshire	SJ 698 502	Insufficient information.
Wytham Wood SSSI	Oxfordshire	SP 462 082	Site selected.
Yarner Wood NNR	Devon	SX 786 789	Site selected.

**Appendix B** – Recording form for ALPHA sampler monitoring. Format follows that of the established recording form used in the NAMN.

<p><b>Ammonia Sampling Record Card - ALPHA Samplers</b></p> <p><b>Site Name:</b> _____</p> <p><b>Exposure Period:</b> _____</p> <p><b>Site Operator:</b> _____</p> <p><b>Samples Set OUT:</b> Date (dd/mm/yy)    /    / Time (GMT 24 hr)    :</p> <p><b>Setting out ALPHA Samplers:</b></p> <ol style="list-style-type: none"> <li>Carefully remove top protective cap and put the top protective cap back into the container.</li> <li>Check that the open membrane cap with the white membrane is securely on the sampler (please avoid touching the membrane when doing this).</li> <li>Attach sampler firmly to velcro on sample holder.</li> </ol> <p><b>Samples Taken IN:</b> Date (dd/mm/yy)    /    / Time (GMT 24 hr)    :</p> <p><b>Collecting in ALPHA Samplers:</b></p> <ol style="list-style-type: none"> <li>To avoid damage to the delicate membranes, put the top protective cap back onto the sampler first, then remove both caps and membrane.</li> <li>Next, put the replacement solid caps provided securely onto the samplers.</li> <li>Return everything to the plastic container.</li> </ol> <p>Please use the reverse of this card to record any comments that may influence the results of the analysis (e.g. equipment problems, nearby land management, livestock etc.).</p> <p>Please enclose this form with the samples in the box supplied.</p>	<p><b>Please indicate if any of the following events occurred during the sampling period:</b></p> <p><b>Manure Spreading</b>    <input type="checkbox"/>    Date _____ Proximity _____</p> <p>Smell:    Faint    <input type="checkbox"/>    Strong    <input type="checkbox"/>    V. Strong    <input type="checkbox"/></p> <p><b>Fertiliser Application</b>    <input type="checkbox"/>    Date _____ Proximity _____</p> <p>Smell:    Faint    <input type="checkbox"/>    Strong    <input type="checkbox"/>    V. Strong    <input type="checkbox"/></p> <p><b>Ploughing of Field</b>    <input type="checkbox"/>    Date _____ Proximity _____</p> <p><b>Cutting of Vegetation</b>    <input type="checkbox"/>    Date _____ Proximity _____</p> <p><b>Burning of Vegetation</b>    <input type="checkbox"/>    Date _____ Proximity _____</p> <p><b>Grazing</b>    Cows    <input type="checkbox"/>    Number _____ Proximity _____</p> <p>Sheep    <input type="checkbox"/>    Number _____ Proximity _____</p> <p>Other    <input type="checkbox"/>    Number _____ Proximity _____</p>
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**Appendix C** – Indicator species lists used in previous studies of nitrogen bio-monitoring in the UK.

<b>Acidophytic (Nitrophobic)</b>	<b>Nitrophyte (Nitrophilic)</b>
<i>Evernia prunastri</i>	<i>Diploica canescens</i>
<i>Hypogymnia</i> sp.	<i>Hyperphyscia adglutinosa</i>
<i>Flavoparmelia caperata</i>	<i>Parmelina</i> sp.
<i>Parmelia saxatilis</i>	<i>Phaeophyscia orbicularis</i>
<i>Parmeliopsis ambigua</i>	<i>Physcia adscendens</i>
<i>Cladonia</i> sp.	<i>Physcia tenella</i>
<i>Platismatia glauca</i>	<i>Physconia</i> sp.
<i>Tuckermanopsis chlorophylla</i>	<i>Xanthoria parietina</i>
<i>Pseudevernia furfuracea</i>	<i>Xanthoria polycarpa</i>
<i>Usnea</i> sp.	

Source: Wolseley, P.A. (2005)

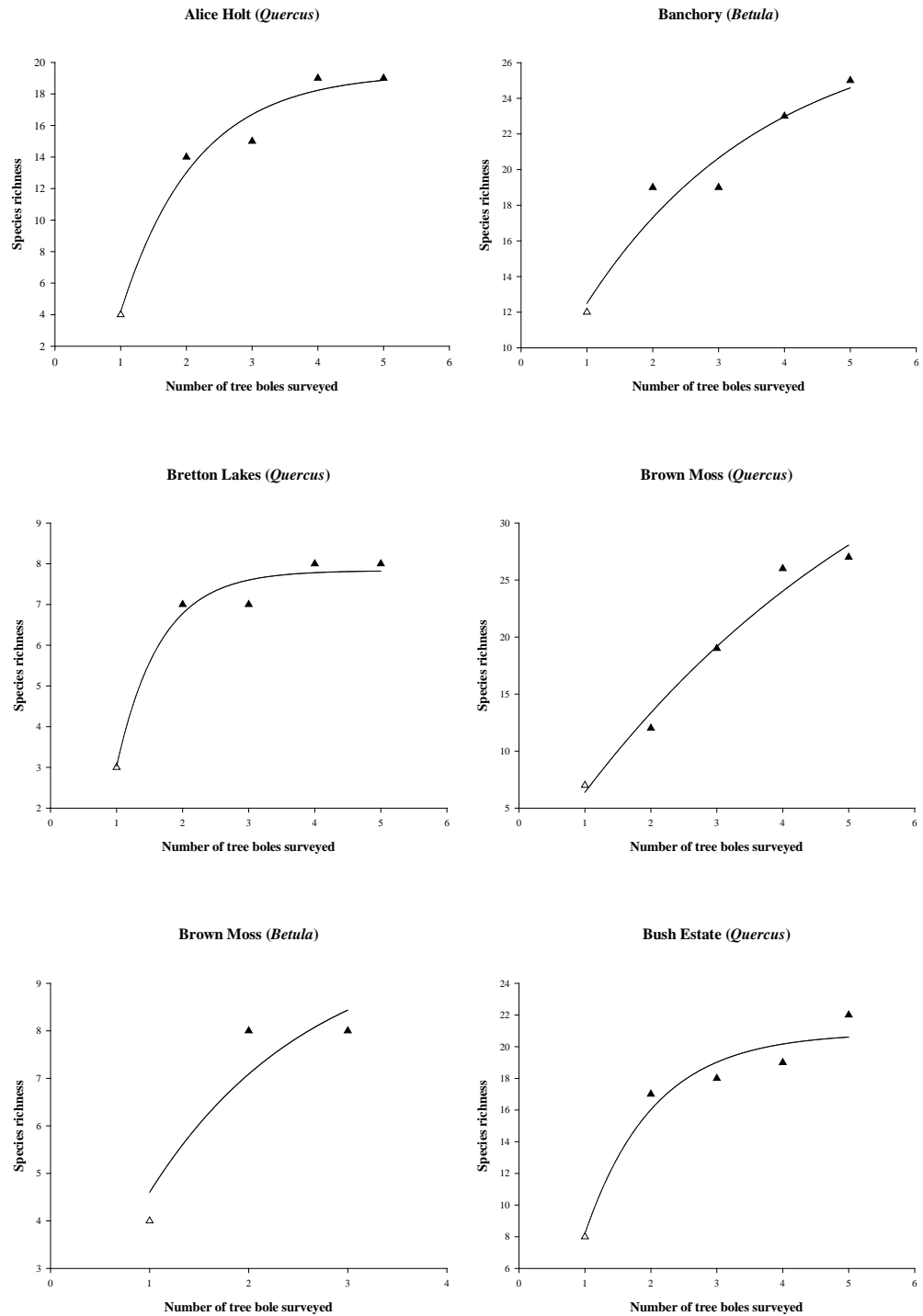
<b>Acidophytic (Nitrophobic)</b>	<b>Nitrophyte (Nitrophilic)</b>
<i>Cladonia</i> sp.	<i>Hyperphyscia adglutinata</i>
<i>Evernia prunastri</i>	<i>Phaeophyscia orbicularis</i>
<i>Flavoparmelia caperata</i>	<i>Physcia adscendens</i>
<i>Hypogymnia</i> spp.	<i>Physcia tenella</i>
<i>Platismatia glauca</i>	<i>Xanthoria candelaria</i>
<i>Pseudevernia furfuracea</i>	<i>Xanthoria parietina</i>
<i>Usnea</i> sp.	<i>Xanthoria polycarpa</i>
<i>Bryoria</i> sp.	

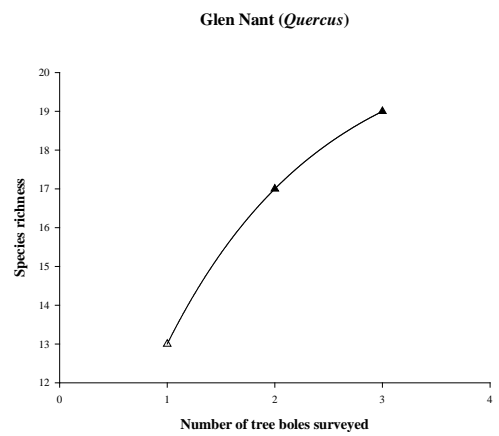
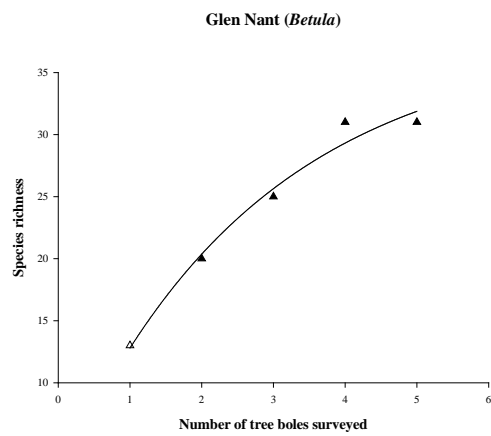
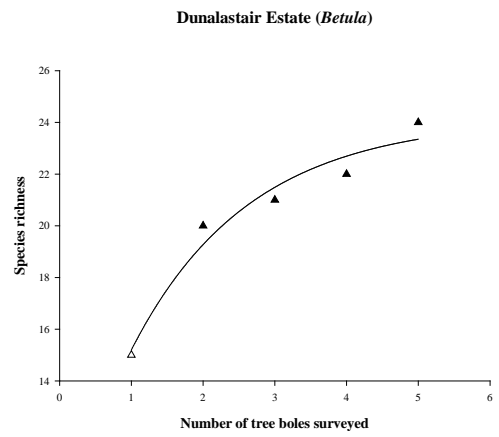
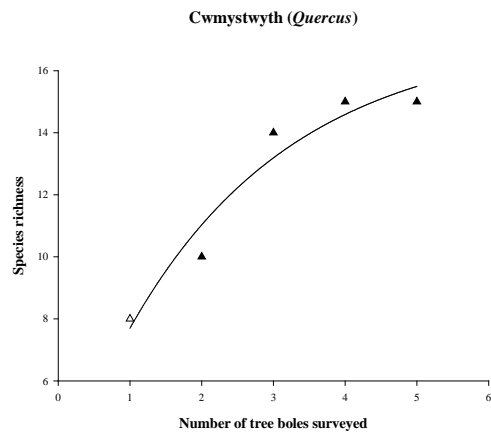
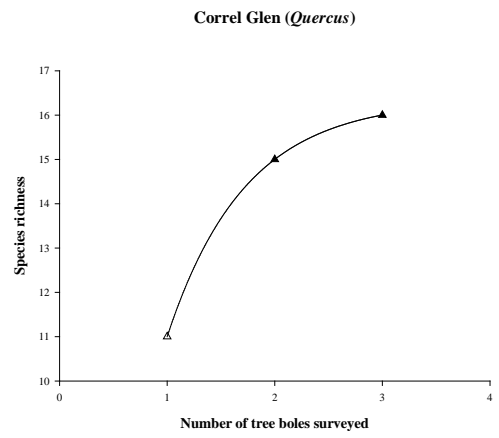
Source: Wolseley, P.A., *et al.* (2009)

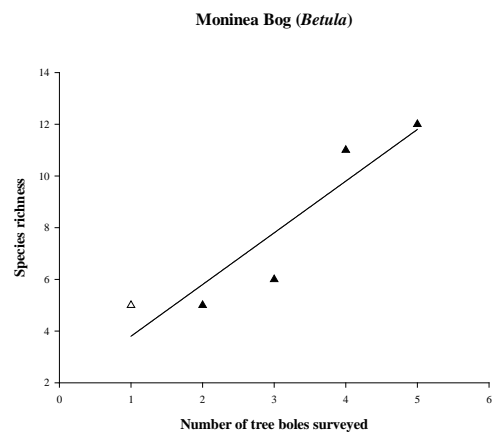
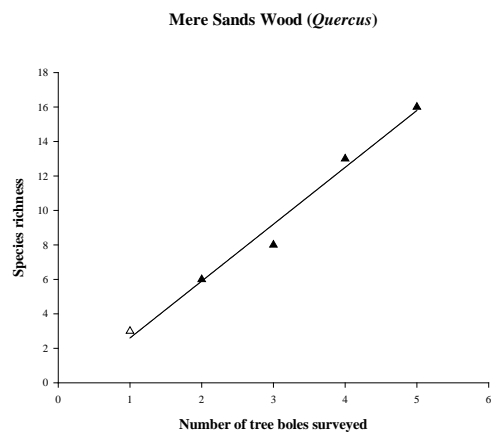
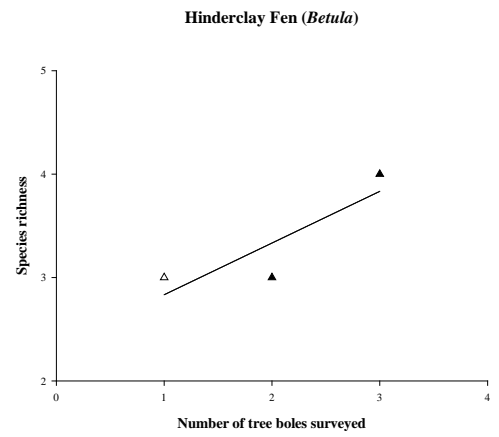
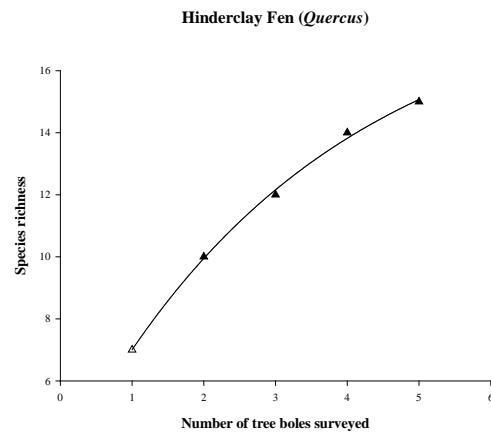
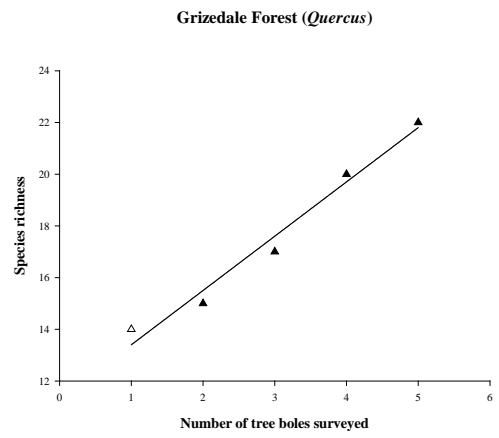
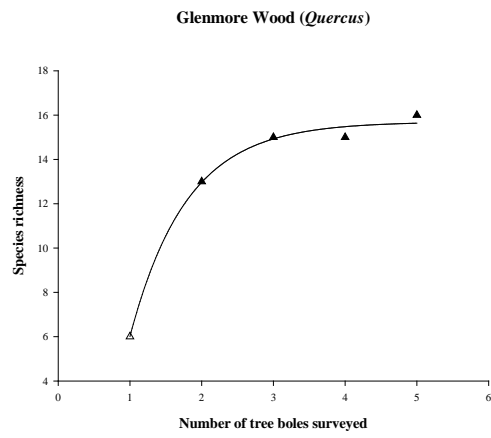
<b>Acidophytic (Nitrophobic)</b>	<b>Nitrophyte (Nitrophilic)</b>
<i>Evernia</i> sp.	<i>Physcia</i> sp.
<i>Hypogymnia</i> sp.	<i>Xanthoria parietina</i>
<i>Usnea</i> sp.	<i>Xanthoria polycarpa</i>

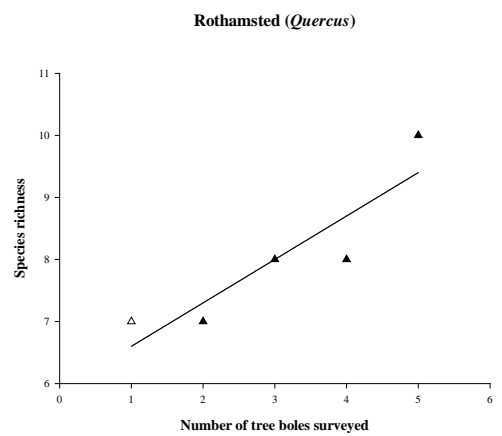
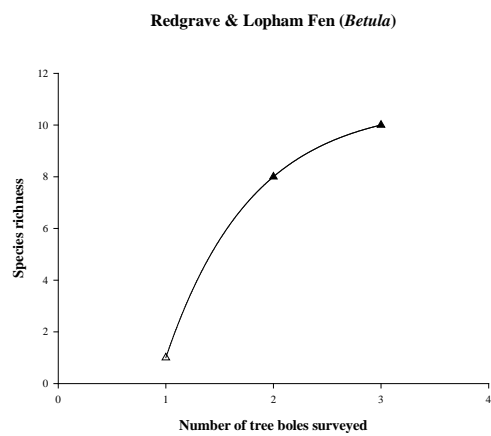
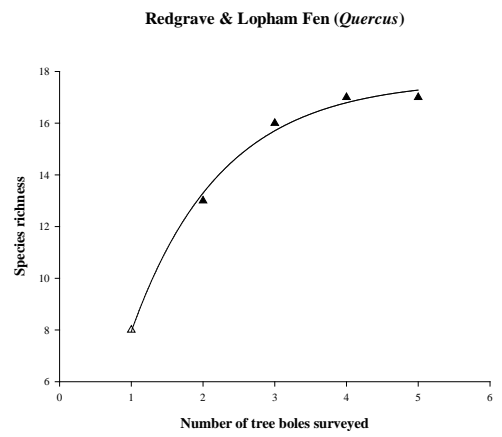
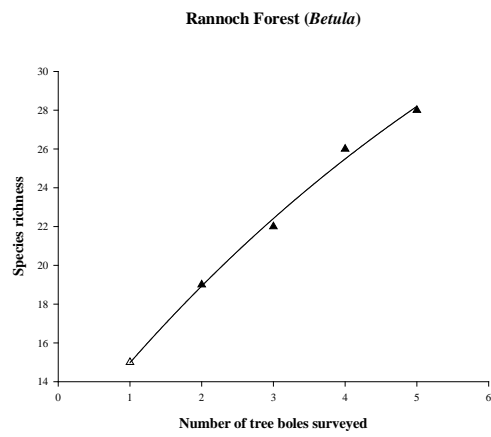
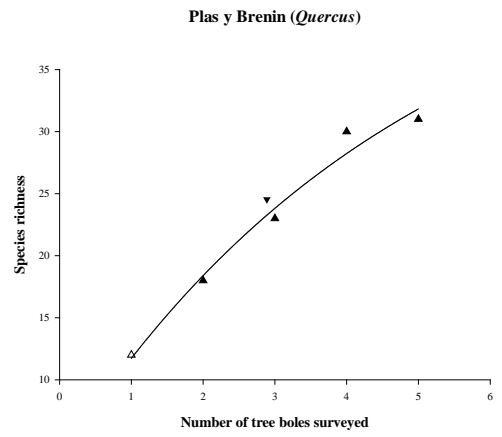
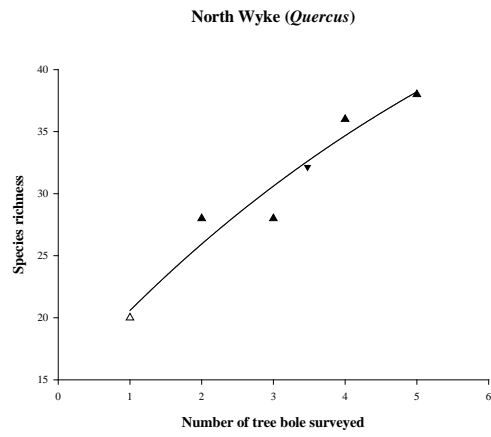
Source: OPAL (2011)

**Appendix D** – Species accumulation curves of epiphytic lichens recorded on tree boles during the regional-scale survey. Best lines fitted using an exponential rise to maximum curve in Sigmaplot 11.0, with linear lines drawn where necessary.

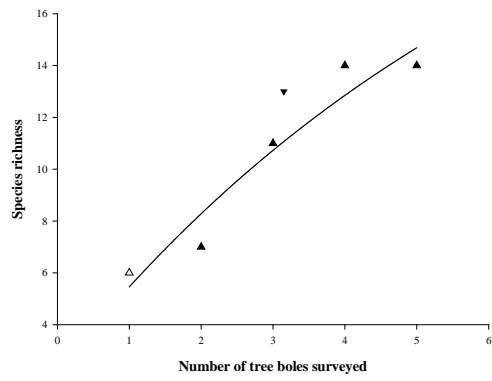




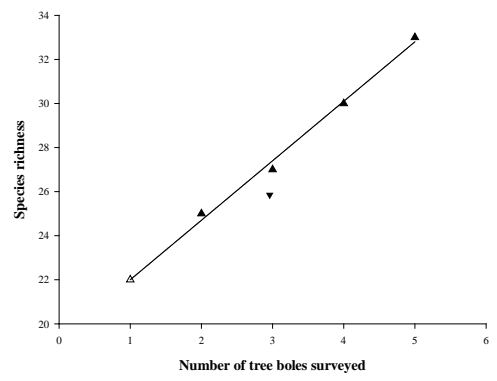




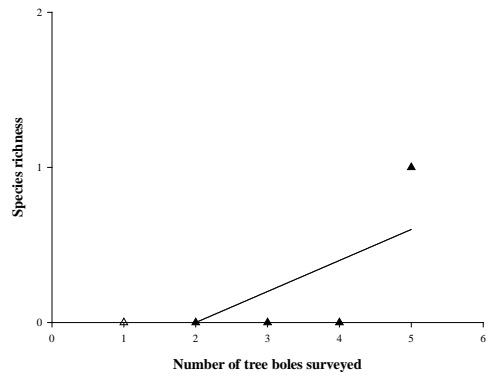
Ruabon (*Betula*)



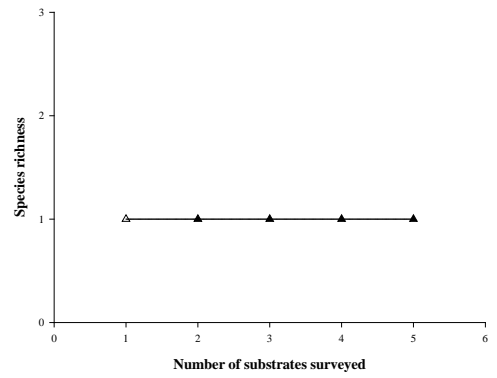
Strath Vaich (*Betula*)



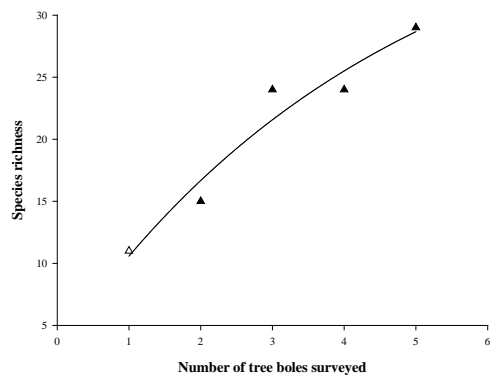
Sutton Bonnington (*Quercus*)



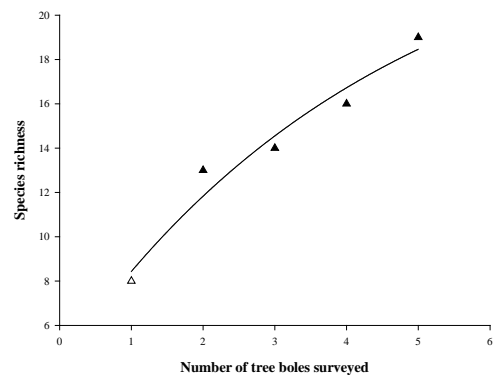
Twentywellsick Wood (*Quercus*)



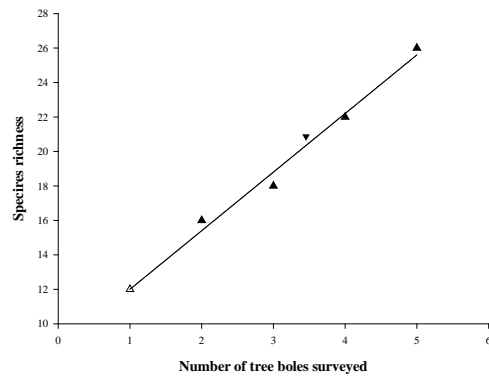
Tycanol Wood (*Quercus*)



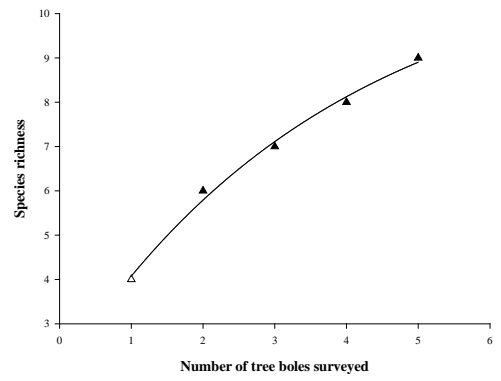
Upper Ballinderry River (*Quercus*)



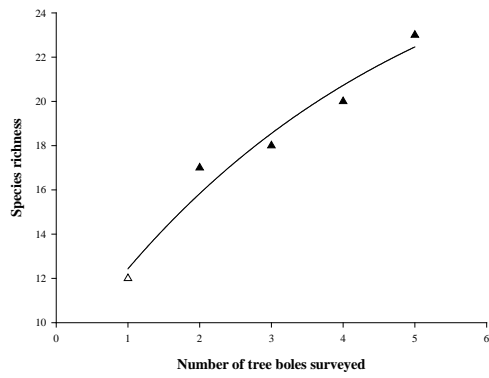
Wood of Cree (*Quercus*)



Wytham Wood (*Quercus*)

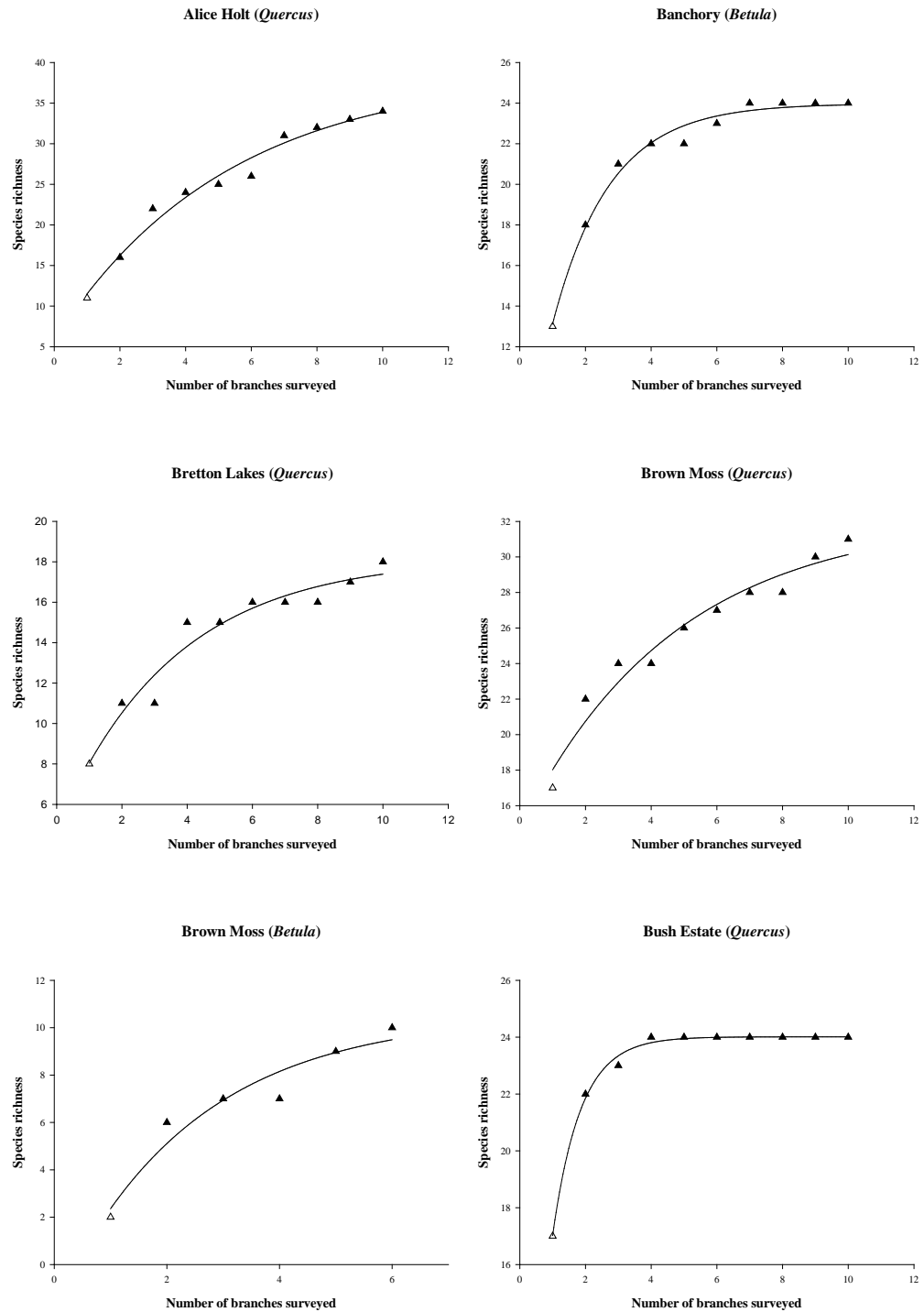


Yarner Wood (*Quercus*)

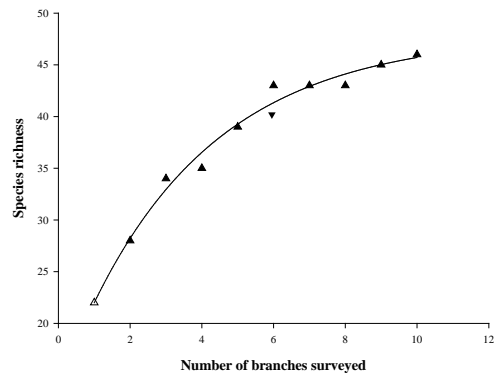




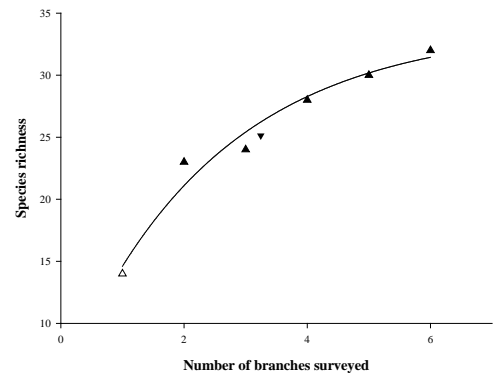
**Appendix E** – Species accumulation curves of epiphytic lichens recorded on tree branches during the regional-scale survey. Best lines fitted using an exponential rise to maximum curve in Sigmaplot 11.0, with exponential growth or sigmoidal lines drawn where necessary.



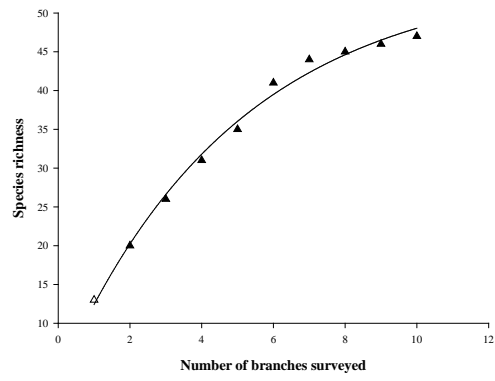
Correl Glen (*Betula*)



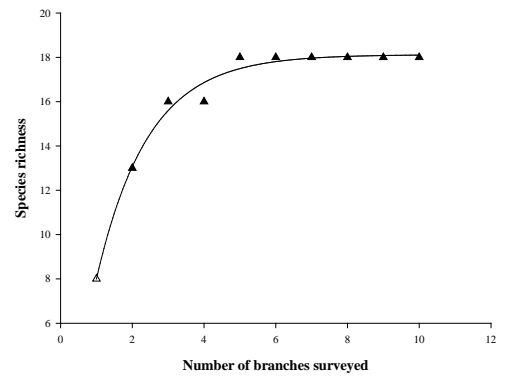
Correl Glen (*Quercus*)



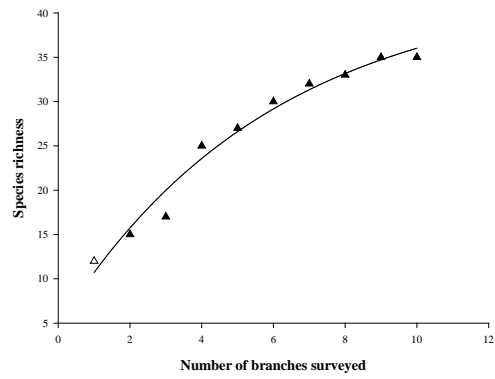
Cwmystwyth (*Quercus*)



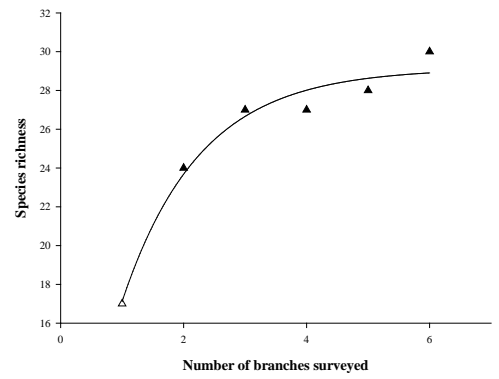
Dunalastair Estate (*Betula*)



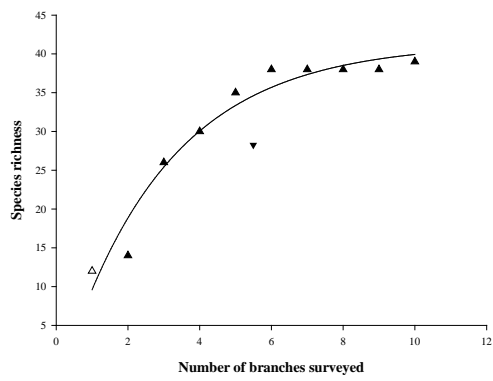
Glen Nant (*Betula*)



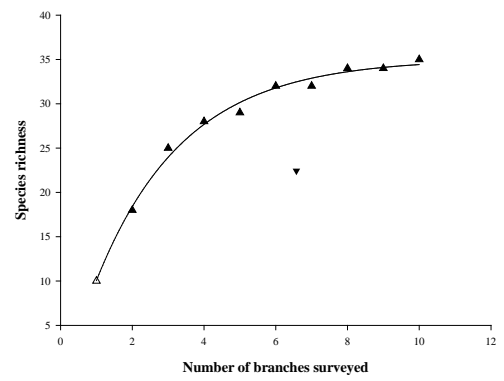
Glen Nant (*Quercus*)



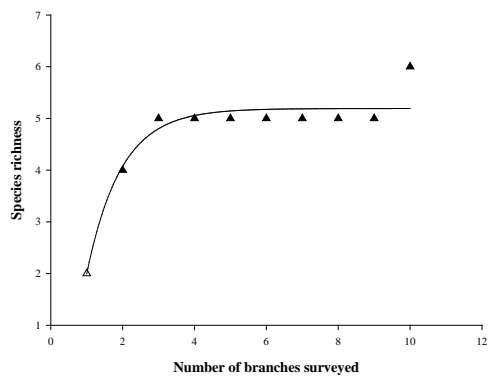
Glenmore Wood (*Quercus*)



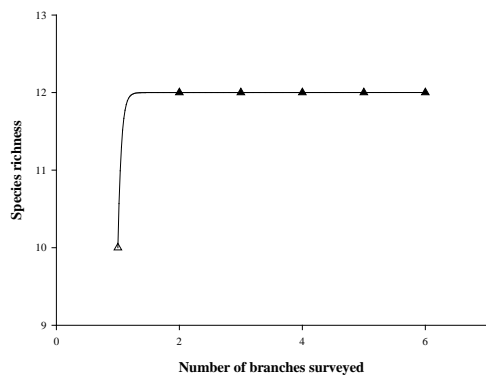
Grizedale Forest (*Quercus*)



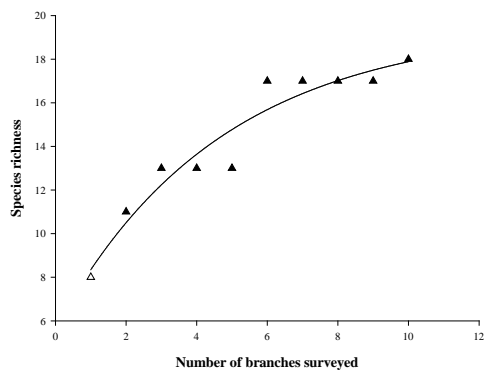
Hinderclay Fen (*Quercus*)



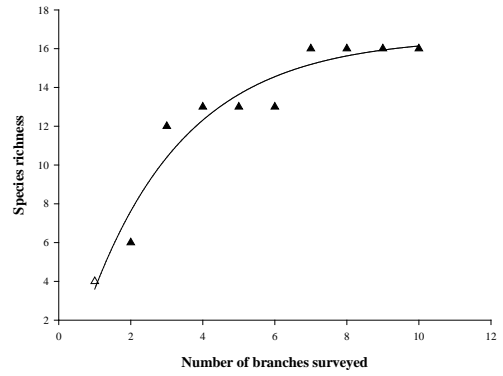
Hinderclay Fen (*Betula*)



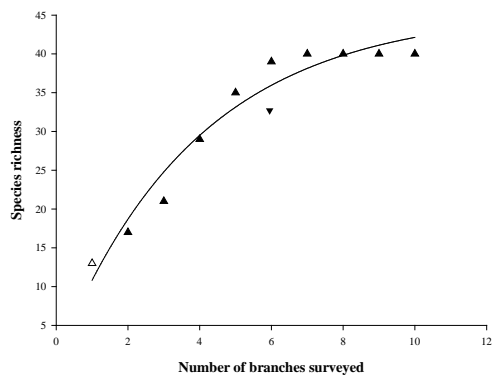
Mere Sands Wood (*Quercus*)



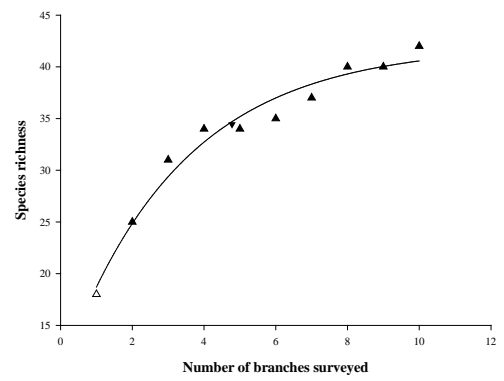
Moninea Bog (*Betula*)



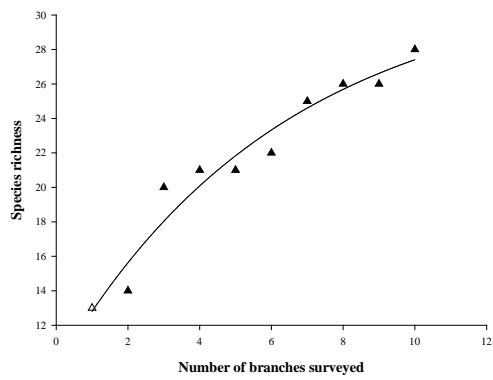
North Wyke (*Quercus*)



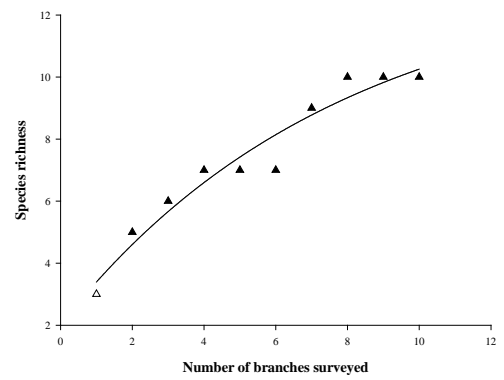
Plas y Brenin (*Quercus*)



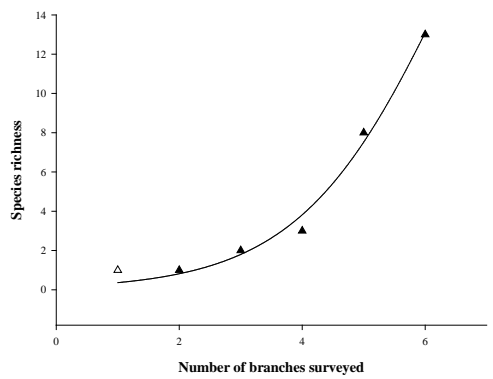
Rannoch Forest (*Betula*)



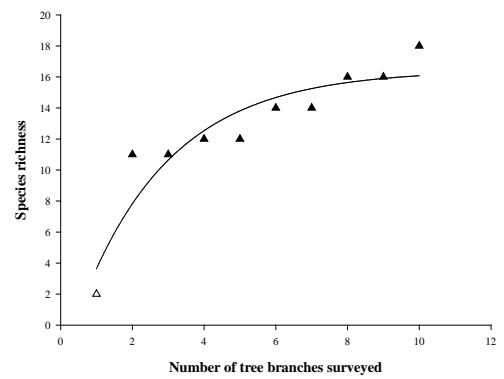
Redgrave & Lopham Fen (*Quercus*)



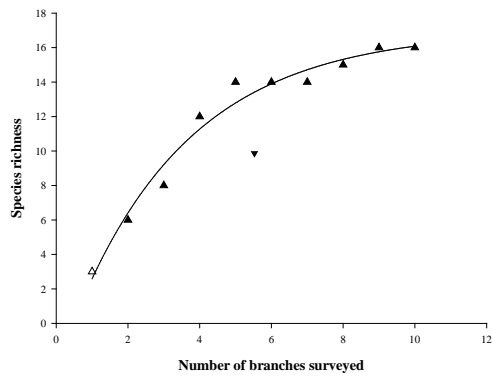
Redgrave & Lopham Fen (*Betula*)



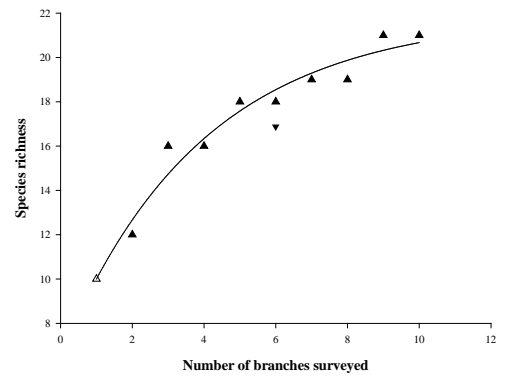
Rothamsted (*Quercus*)



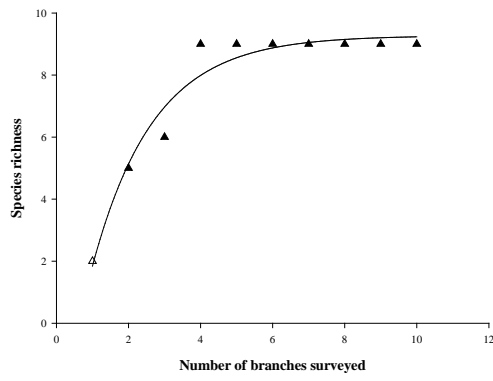
Ruabon (*Betula*)



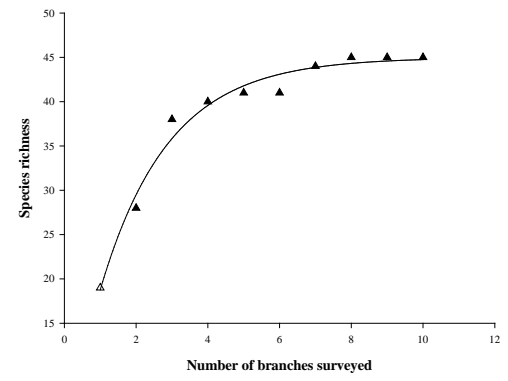
Strath Vaich (*Betula*)



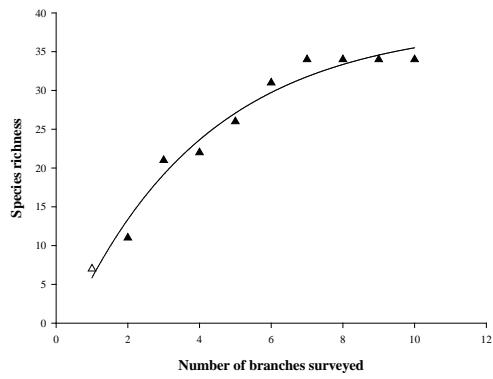
Sutton Bonnington (*Quercus*)



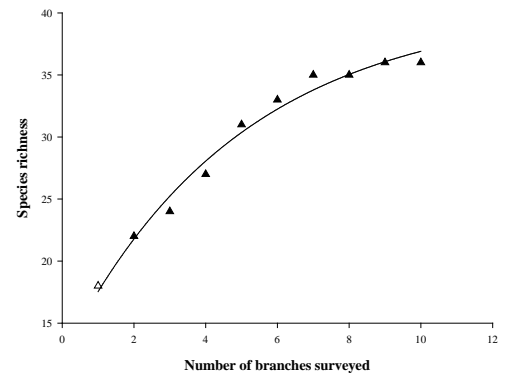
Tycanol Wood (*Quercus*)

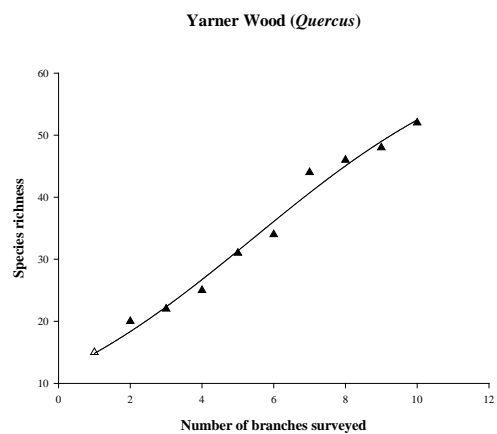
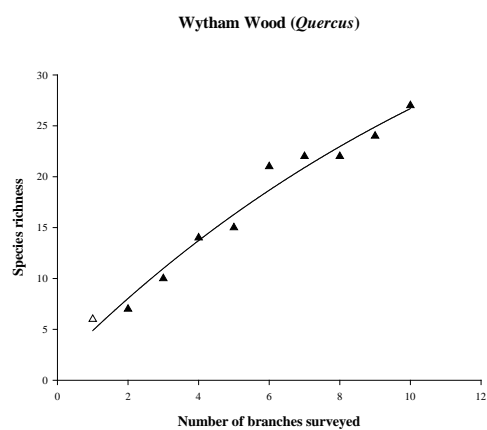


Upper Ballinderry River (*Quercus*)



Wood of Cree (*Quercus*)





**Appendix F** – List of species recorded during the regional-scale survey, frequency of occurrence on trunks and branches and host phorophyte species.

Epiphytic Species	Number of Sites at which Recorded				Phorophyte Species
	Total	Trunks	Branches	Both	
<i>Amandinea punctata</i>	8	4	5	1	<i>Betula &amp; Quercus</i>
<i>Amandinea</i> sp.	1	0	1	0	<i>Quercus</i>
<i>Anisomeridium biforme</i>	7	2	6	1	<i>Quercus</i>
<i>Anisomeridium polypori</i>	1	1	0	0	<i>Quercus</i>
<i>Anisomeridium ranunculosporum</i>	3	2	1	0	<i>Quercus</i>
<i>Arthonia cinnabarina</i>	3	2	2	1	<i>Quercus</i>
<i>Arthonia didyma</i>	2	0	2	0	<i>Betula</i>
<i>Arthonia elegans</i>	3	2	1	0	<i>Quercus</i>
<i>Arthonia punctiformis</i>	13	2	12	1	<i>Betula &amp; Quercus</i>
<i>Arthonia radiata</i>	15	2	14	1	<i>Betula &amp; Quercus</i>
<i>Arthonia spadicea</i>	4	4	0	0	<i>Quercus</i>
<i>Arthonia</i> sp.	3	3	0	0	<i>Quercus</i>
<i>Arthonia vinosa</i>	2	2	0	0	<i>Quercus</i>
<i>Arthopyrenia analepta</i>	4	0	4	0	<i>Betula &amp; Quercus</i>
<i>Arthopyrenia punctiformis</i>	4	0	4	0	<i>Quercus</i>
<i>Arthopyrenia</i> sp.	3	1	3	1	<i>Quercus</i>
<i>Bacidia adastrata</i>	1	0	1	0	<i>Quercus</i>
<i>Bacidia arceutina</i>	1	0	1	0	<i>Quercus</i>
<i>Bryoria fuscescens</i>	6	4	4	2	<i>Betula</i>
<i>Buellia disciformis</i>	2	0	2	0	<i>Betula &amp; Quercus</i>
<i>Buellia erubescens</i>	3	2	1	0	<i>Betula &amp; Quercus</i>
<i>Buellia griseovirens</i>	5	4	3	2	<i>Betula &amp; Quercus</i>
<i>Bundophoron melanocarpum</i>	1	1	0	0	<i>Betula</i>
<i>Calicium viride</i>	4	4	0	0	<i>Betula &amp; Quercus</i>
<i>Candelariella reflexa</i>	13	4	11	2	<i>Betula &amp; Quercus</i>
<i>Candelariella vitellina</i>	1	1	0	0	<i>Quercus</i>
<i>Candelariella xanthostigma</i>	1	1	0	0	<i>Quercus</i>
<i>Cetraria aculeata</i>	1	1	0	0	<i>Betula</i>
<i>Cetraria sepicola</i>	2	0	2	0	<i>Betula</i>
<i>Cetrelia olivetorum</i> s.l.	1	1	1	1	<i>Quercus</i>
<i>Chaenotheca bruneola</i>	1	1	0	0	<i>Quercus</i>
<i>Chaenotheca chrysocephala</i>	1	1	0	0	<i>Betula</i>
<i>Chaenotheca ferruginea</i>	4	4	0	0	<i>Betula &amp; Quercus</i>
<i>Chrysothrix candelaris</i>	4	4	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia chlorophaea</i>	2	2	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia coniocraea</i>	9	9	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia diversa</i>	2	2	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia polydactyla</i>	7	7	2	2	<i>Betula &amp; Quercus</i>
<i>Cladonia pyxidata</i>	5	5	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia squamosa</i>	3	3	0	0	<i>Betula &amp; Quercus</i>
<i>Cladonia</i> sp.	4	4	0	0	<i>Quercus</i>
<i>Cliostomum griffithii</i>	14	14	0	0	<i>Betula &amp; Quercus</i>
<i>Cyrtidula quercus</i>	17	0	17	0	<i>Quercus</i>
<i>Dimerella lutea</i>	6	6	1	1	<i>Betula &amp; Quercus</i>
<i>Dimerella pineti</i>	6	5	2	1	<i>Betula &amp; Quercus</i>
<i>Diploica canescens</i>	2	2	0	0	<i>Quercus</i>
<i>Enterographa crassa</i>	1	1	0	0	<i>Quercus</i>
<i>Evernia prunastri</i>	21	12	17	8	<i>Betula &amp; Quercus</i>
<i>Flavoparmelia caperata</i>	11	9	6	4	<i>Betula &amp; Quercus</i>
<i>Fuscidea lightfootii</i>	23	4	22	3	<i>Betula &amp; Quercus</i>

Epiphytic Species	Number of Sites at which Recorded				Phorophyte Species
Lichens	Total	Trunks	Branches	Both	
<i>Graphina anguina</i>	2	1	2	1	<i>Betula</i> & <i>Quercus</i>
<i>Graphis elegans</i>	12	1	12	1	<i>Betula</i> & <i>Quercus</i>
<i>Graphis scripta</i>	13	1	12	0	<i>Betula</i> & <i>Quercus</i>
<i>Hypocenomyce scalaris</i>	1	1	0	0	<i>Quercus</i>
<i>Hypogymnia physodes</i>	18	11	18	11	<i>Betula</i> & <i>Quercus</i>
<i>Hypogymnia tubulosa</i>	16	1	16	1	<i>Betula</i> & <i>Quercus</i>
<i>Hypogymnia</i> sp.	2	0	2	0	<i>Quercus</i>
<i>Hypotrachyna laevigata</i>	5	5	2	2	<i>Betula</i> & <i>Quercus</i>
<i>Hypotrachyna revoluta</i>	16	9	14	7	<i>Betula</i> & <i>Quercus</i>
<i>Hypotrachyna sinuosa</i>	2	0	2	0	<i>Betula</i>
<i>Hypotrachyna taylorensis</i>	2	2	1	1	<i>Quercus</i>
<i>Imshaugia aleurites</i>	1	1	0	0	<i>Betula</i>
<i>Jamesiella anastomosans</i>	1	1	0	0	<i>Betula</i>
<i>Japewiella tavaresiana</i>	4	0	4	0	<i>Quercus</i>
<i>Lecanactis abietina</i>	2	2	0	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecania cyrtella</i>	2	0	2	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecania naegelii</i>	2	0	2	0	<i>Quercus</i>
<i>Lecanora alboflavida</i>	4	2	2	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora carpinea</i>	7	0	7	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora chlarotera</i>	27	12	27	12	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora compallens</i>	5	3	2	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora confusa</i>	6	3	6	3	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora dispersa</i>	1	0	1	0	<i>Quercus</i>
<i>Lecanora expallens</i>	15	14	7	6	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora persimilis</i>	8	1	7	0	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora pulicaris</i>	14	3	14	3	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora symmicta</i>	14	1	14	1	<i>Betula</i> & <i>Quercus</i>
<i>Lecanora</i> sp.	3	0	3	0	<i>Quercus</i>
<i>Lecidella elaeochroma</i>	25	9	22	6	<i>Betula</i> & <i>Quercus</i>
<i>Lepraria incana</i>	19	19	6	6	<i>Betula</i> & <i>Quercus</i>
<i>Lepraria lobificans</i>	13	12	6	5	<i>Betula</i> & <i>Quercus</i>
<i>Lepraria rigidula</i>	8	5	4	1	<i>Betula</i> & <i>Quercus</i>
<i>Lepraria</i> sp.	1	1	0	0	<i>Quercus</i>
<i>Leptorhaphis epidermidis</i>	4	3	2	1	<i>Betula</i>
<i>Lobaria pulmonaria</i>	1	1	0	0	<i>Betula</i>
<i>Megalaria pulverea</i>	3	3	1	1	<i>Betula</i> & <i>Quercus</i>
<i>Melanelixia fuliginosa</i> subsp. <i>glabratula</i>	15	10	11	6	<i>Betula</i> & <i>Quercus</i>
<i>Melanelixia subaurifera</i>	22	5	21	4	<i>Betula</i> & <i>Quercus</i>
<i>Melanohalea elegantula</i>	2	0	2	0	<i>Quercus</i>
<i>Melanohalea exasperata</i>	2	0	2	0	<i>Quercus</i>
<i>Melanohalea exasperatula</i>	3	0	3	0	<i>Quercus</i>
<i>Melanohalea lacinatula</i>	2	0	2	0	<i>Quercus</i>
<i>Melanohalea</i> sp.	1	0	1	0	<i>Quercus</i>
<i>Menegazzia terebrata</i>	1	1	1	1	<i>Betula</i>
<i>Micarea nitschkeana</i>	1	0	1	0	<i>Quercus</i>
<i>Mycoblastus affinis</i>	1	1	0	0	<i>Betula</i>
<i>Mycoblastus caesius</i>	2	2	1	1	<i>Betula</i> & <i>Quercus</i>
<i>Mycoblastus fucatus</i>	9	4	7	2	<i>Betula</i> & <i>Quercus</i>
<i>Mycoblastus sanguinarius</i>	3	3	1	1	<i>Betula</i>
<i>Mycoporum antecellens</i>	7	0	7	0	<i>Betula</i> & <i>Quercus</i>



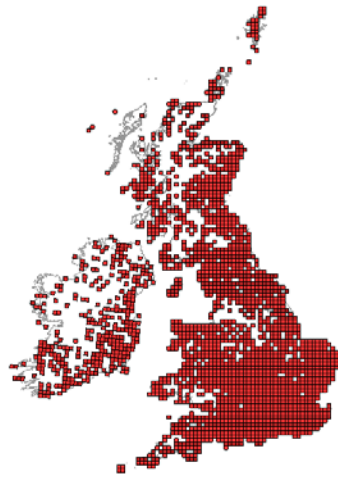
Epiphytic Species	Number of Sites at which Recorded				Phorophyte Species
	Total	Trunks	Branches	Both	
<i>Normandina pulchella</i>	8	7	6	5	<i>Betula &amp; Quercus</i>
<i>Ochrolechia androgyna</i>	8	7	1	0	<i>Betula &amp; Quercus</i>
<i>Ochrolechia microstictoides</i>	2	0	2	0	<i>Betula</i>
<i>Ochrolechia</i> sp.	1	1	0	0	<i>Quercus</i>
<i>Opegrapha atra</i>	6	2	6	2	<i>Betula &amp; Quercus</i>
<i>Opegrapha herbarum</i>	3	2	2	1	<i>Quercus</i>
<i>Opegrapha varia</i>	1	1	0	0	<i>Quercus</i>
<i>Opegrapha vulgata</i>	3	2	2	1	<i>Betula &amp; Quercus</i>
<i>Parmelia saxatilis</i>	16	12	11	7	<i>Betula &amp; Quercus</i>
<i>Parmelia sulcata</i>	24	12	22	10	<i>Betula &amp; Quercus</i>
<i>Parmelia</i> sp.	7	2	5	0	<i>Betula &amp; Quercus</i>
<i>Parmotrema perlatum</i>	9	4	7	2	<i>Betula &amp; Quercus</i>
<i>Parmotrema reticulatum</i>	3	0	3	0	<i>Betula &amp; Quercus</i>
<i>Pertusaria albescens</i> var. <i>albescens</i>	8	4	5	1	<i>Betula &amp; Quercus</i>
<i>Pertusaria albescens</i> var. <i>corallina</i>	6	6	2	2	<i>Betula &amp; Quercus</i>
<i>Pertusaria amara</i>	4	3	2	1	<i>Betula &amp; Quercus</i>
<i>Pertusaria coccodes</i>	2	0	2	0	<i>Quercus</i>
<i>Pertusaria hemisphaerica</i>	3	2	1	0	<i>Betula &amp; Quercus</i>
<i>Pertusaria hymenea</i>	4	4	0	0	<i>Quercus</i>
<i>Pertusaria leioplaca</i>	3	1	2	0	<i>Quercus</i>
<i>Pertusaria pertusa</i>	6	5	3	2	<i>Betula &amp; Quercus</i>
<i>Pertusaria pupillaris</i>	1	1	0	0	<i>Betula</i>
<i>Phaeographis dendritica</i>	1	0	1	0	<i>Quercus</i>
<i>Phaeographis smithii</i>	5	1	4	0	<i>Betula &amp; Quercus</i>
<i>Phlyctis argena</i>	14	2	14	2	<i>Betula &amp; Quercus</i>
<i>Physcia adscendens</i>	9	3	6	0	<i>Betula &amp; Quercus</i>
<i>Physcia aipolia</i>	4	0	4	0	<i>Quercus</i>
<i>Physcia leptalea</i>	2	1	1	0	<i>Quercus</i>
<i>Physcia stellaris</i>	4	0	4	0	<i>Quercus</i>
<i>Physcia tenella</i>	20	5	20	5	<i>Betula &amp; Quercus</i>
<i>Physcia</i> sp.	2	0	2	0	<i>Betula &amp; Quercus</i>
<i>Platismatia glauca</i>	14	8	13	7	<i>Betula &amp; Quercus</i>
<i>Porina aenea</i>	7	0	7	0	<i>Quercus</i>
<i>Pseudevernia furfuracea</i>	7	2	7	2	<i>Betula &amp; Quercus</i>
<i>Punctelia borleri</i>	2	0	2	0	<i>Quercus</i>
<i>Punctelia jeckeri</i>	4	0	4	0	<i>Betula &amp; Quercus</i>
<i>Punctelia reddenda</i>	4	1	3	0	<i>Betula &amp; Quercus</i>
<i>Punctelia subrudecta</i>	11	4	11	4	<i>Betula &amp; Quercus</i>
<i>Pyrrhospora quernea</i>	6	6	0	0	<i>Quercus</i>
<i>Ramalina calicaris</i>	1	0	1	0	<i>Quercus</i>
<i>Ramalina canariensis</i>	4	2	3	1	<i>Quercus</i>
<i>Ramalina farinacea</i>	19	8	16	5	<i>Betula &amp; Quercus</i>
<i>Ramalina fastigata</i>	4	1	4	1	<i>Betula &amp; Quercus</i>
<i>Ramalina fraxinea</i>	5	0	5	0	<i>Quercus</i>
<i>Ramalina</i> sp.	10	3	8	1	<i>Quercus</i>
<i>Rinodina sophodes</i>	3	0	3	0	<i>Betula</i>
<i>Schismatomma decolourans</i>	1	1	0	0	<i>Quercus</i>
<i>Schismatomma niveum</i>	1	1	0	0	<i>Quercus</i>
<i>Scoliciosporum chlorococcum</i>	18	3	18	3	<i>Betula &amp; Quercus</i>
<i>Sphaerophorus globosus</i>	5	5	0	0	<i>Betula &amp; Quercus</i>

Epiphytic Species	Number of Sites at which Recorded				Phorophyte Species
Lichens	Total	Trunks	Branches	Both	
<i>Sticta limbata</i>	1	1	0	0	<i>Quercus</i>
<i>Thelotrema lepadinum</i>	9	7	4	2	<i>Betula</i> & <i>Quercus</i>
<i>Trapelia corticola</i>	1	1	0	0	<i>Quercus</i>
<i>Trapeliopsis pseudogranulosa</i>	1	1	0	0	<i>Betula</i>
<i>Tuckermanopsis chlorophylla</i>	4	4	0	0	<i>Betula</i>
<i>Usnea cornuta</i>	7	2	7	2	<i>Betula</i> & <i>Quercus</i>
<i>Usnea filipendula</i>	4	1	4	1	<i>Betula</i> & <i>Quercus</i>
<i>Usnea flammea</i>	8	6	6	4	<i>Betula</i> & <i>Quercus</i>
<i>Usnea hirta</i>	4	1	4	1	<i>Betula</i> & <i>Quercus</i>
<i>Usnea rubicunda</i>	1	1	0	0	<i>Betula</i> & <i>Quercus</i>
<i>Usnea subfloridana</i>	10	6	10	6	<i>Betula</i> & <i>Quercus</i>
<i>Usnea wasmuthii</i>	6	2	6	2	<i>Betula</i> & <i>Quercus</i>
<i>Usnea</i> sp.	11	4	10	3	<i>Betula</i> & <i>Quercus</i>
<i>Xanthoria candelaria</i> agg.	11	4	11	4	<i>Betula</i> & <i>Quercus</i>
<i>Xanthoria parietina</i>	18	6	18	6	<i>Betula</i> & <i>Quercus</i>
<i>Xanthoria polycarpa</i>	13	6	13	6	<i>Betula</i> & <i>Quercus</i>
<b>Bryophyta</b>					
<i>Atrichum undulatum</i>	1	1	0	0	<i>Quercus</i>
<i>Dicranella heteromalla</i>	1	1	0	0	<i>Betula</i>
<i>Dicranum scoparium</i>	11	11	1	1	<i>Betula</i> & <i>Quercus</i>
<i>Hypnum cupressiforme</i>	12	11	4	3	<i>Betula</i> & <i>Quercus</i>
<i>Isoetecium myosuroides</i>	18	17	10	9	<i>Betula</i> & <i>Quercus</i>
<i>Mnium hornum</i>	1	1	0	0	<i>Quercus</i>
<i>Orthotrichum affine</i>	7	2	5	0	<i>Betula</i> & <i>Quercus</i>
<i>Rhytidiadelphus loreus</i>	2	2	0	0	<i>Betula</i> & <i>Quercus</i>
<i>Thuidium tamariscinum</i>	1	1	0	0	<i>Quercus</i>
<i>Ulota crispa</i>	18	8	12	2	<i>Betula</i> & <i>Quercus</i>
<b>Marchantiophyta</b>					
<i>Calypogeia fissa</i>	6	5	1	0	<i>Betula</i> & <i>Quercus</i>
<i>Frullania dilatata</i>	12	8	10	6	<i>Betula</i> & <i>Quercus</i>
<i>Frullania tamarisci</i>	7	5	3	1	<i>Betula</i> & <i>Quercus</i>
<i>Lophocolea bidentata</i>	2	2	0	0	<i>Betula</i> & <i>Quercus</i>
<i>Lophocolea heterophylla</i>	3	2	1	0	<i>Betula</i> & <i>Quercus</i>
<i>Metzgeria furcata</i>	9	4	7	2	<i>Betula</i> & <i>Quercus</i>

**Appendix G** Species used in the main matrices for trunks and branches together with the species codes used in the NMDS ordination analyses.

Lichen Species	NMDS Code	Lichen Species	NMDS Code
<i>Amandinea punctata</i>	Am1	<i>Opegrapha atra</i>	Op1
<i>Anisomeridium biforme</i>	An1	<i>Parmelia saxatilis</i>	Pm1
<i>Arthonia punctiformis</i>	At1	<i>Parmelia sulcata</i>	Pm2
<i>Arthonia radiata</i>	At2	<i>Parmotrema perlatum</i>	Pt1
<i>Arthonia spadicea</i>	At3	<i>Parmotrema reticulatum</i>	Pt2
<i>Arthopyrenia analepta</i>	Ap1	<i>Pertusaria albescens</i> var. <i>albescens</i>	Pe1
<i>Arthopyrenia punctiformis</i>	Ap2	<i>Pertusaria albescens</i> var. <i>corallina</i>	Pe2
<i>Bryoria fuscescens</i>	Br1	<i>Pertusaria amara</i>	Pe3
<i>Buellia griseovirens</i>	Bu1	<i>Pertusaria hymenea</i>	Pe4
<i>Calicium viride</i>	Ca1	<i>Pertusaria pertusa</i>	Pe5
<i>Candelariella reflexa</i>	Ce1	<i>Phaeographis smithii</i>	Pg1
<i>Chaenotheca ferruginea</i>	Ch1	<i>Phlyctis argena</i>	Pc1
<i>Chrysothrix candelaris</i>	Cx1	<i>Physcia adscendens</i>	Ph1
<i>Cladonia coniocraea</i>	Cl1	<i>Physcia aipolia</i>	Ph2
<i>Cladonia polydactyla</i>	Cl2	<i>Physcia stellaris</i>	Ph3
<i>Cladonia pyxidata</i>	Cl3	<i>Physcia tenella</i>	Ph4
<i>Cladonia squamosa</i>	Cl4	<i>Platismatia glauca</i>	Pl1
<i>Cliostomum griffithii</i>	Cm1	<i>Porina aenea</i>	Po1
<i>Cyrtidula quercus</i>	Cy1	<i>Pseudevernia furfuracea</i>	Ps1
<i>Dimerella lutea</i>	Dm1	<i>Punctelia jeckeri</i>	Pu1
<i>Dimerella pineti</i>	Dm2	<i>Punctelia reddenda</i>	Pu2
<i>Evernia prunastri</i>	Ev1	<i>Punctelia subrudecta</i>	Pu3
<i>Flavoparmelia caperata</i>	Fl1	<i>Pyrrhospora quemea</i>	Py1
<i>Fuscidea lightfootii</i>	Fu1	<i>Ramalina canariensis</i>	Ra1
<i>Graphis elegans</i>	Gr1	<i>Ramalina farinacea</i>	Ra2
<i>Graphis scripta</i>	Gr2	<i>Ramalina fastigiata</i>	Ra3
<i>Hypogymnia physodes</i>	Hy1	<i>Ramalina fraxinea</i>	Ra4
<i>Hypogymnia tubulosa</i>	Hy2	<i>Rinodina sophodes</i>	Ri1
<i>Hypotrachyna laevigata</i>	Hn1	<i>Scoliciosporum chlorococcum</i>	Sc1
<i>Hypotrachyna revoluta</i>	Hn2	<i>Sphaerophorus globosus</i>	Sp1
<i>Japewiella tavaresiana</i>	Ja1	<i>Thelotrema lepadinum</i>	Th1
<i>Lecanora carpinea</i>	La1	<i>Tuckermanopsis chlorophylla</i>	Tu1
<i>Lecanora chlorotera</i>	La2	<i>Usnea cornuta</i>	Us1
<i>Lecanora compallens</i>	La3	<i>Usnea filipendula</i>	Us2
<i>Lecanora confusa</i>	La4	<i>Usnea flammea</i>	Us3
<i>Lecanora expallens</i>	La5	<i>Usnea hirta</i>	Us4
<i>Lecanora persimilis</i>	La6	<i>Usnea subfloridana</i>	Us5
<i>Lecanora pulicaris</i>	La7	<i>Usnea wasmuthii</i>	Us6
<i>Lecanora symmicta</i>	La8	<i>Xanthoria candelaria</i> s.l.	Xa1
<i>Lecidella elaeochroma</i>	Li1	<i>Xanthoria parietina</i>	Xa2
<i>Lepraria incana</i>	Lp1	<i>Xanthoria polycarpa</i>	Xa3
<i>Lepraria lobificans</i>	Lp2	<b>Bryophyte Species</b>	<b>NMDS Code</b>
<i>Lepraria rigidula</i>	Lp3		
<i>Leptorhaphis epidermidis</i>	Lt1		
<i>Megalania pulvereae</i>	Mg1	<i>Calypogeia fissa</i>	Co1
<i>Melanelixia fuliginosa</i> subsp. <i>glabratula</i>	Mx1	<i>Dicranum scoparium</i>	Dc1
<i>Melanelixia subaurifera</i>	Mx2	<i>Frullania dilatata</i>	Fr1
<i>Melanohalea exasperatula</i>	Mh1	<i>Frullania tamarisci</i>	Fr2
<i>Mycoblastus fucatus</i>	Mb1	<i>Hypnum cupressiforme</i>	Hm1
<i>Mycoblastus sanguinarius</i>	Mb2	<i>Isothecium myosuroides</i>	Is1
<i>Mycoporum antecellens</i>	Mp1	<i>Metzgeria furcata</i>	Mt1
<i>Normanidina pulchella</i>	No1	<i>Orthotrichum affine</i>	Or1
<i>Ochrolechia androgyna</i>	Oc1	<i>Ulota crispa</i>	Ul1

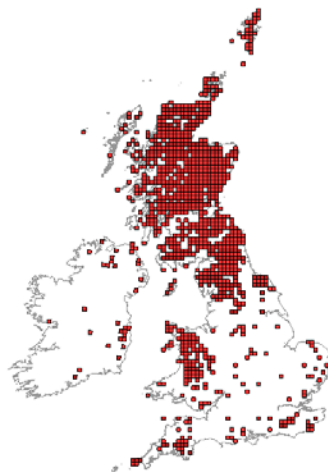
**Appendix H** 10 km square D-maps of the species shortlisted as indicator species for the scoring system in the thesis. Data source: [NBN Gateway \(2011\)](#).



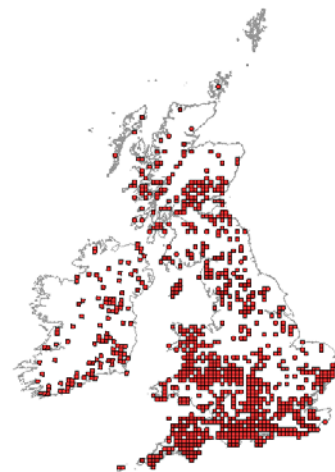
*Amandinea punctata*



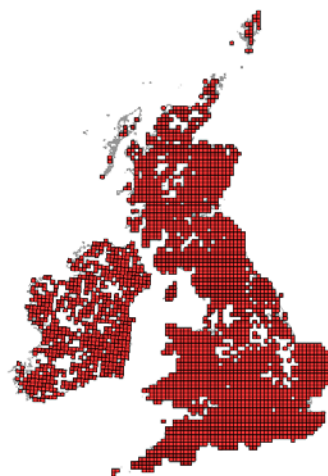
*Arthonia radiata*



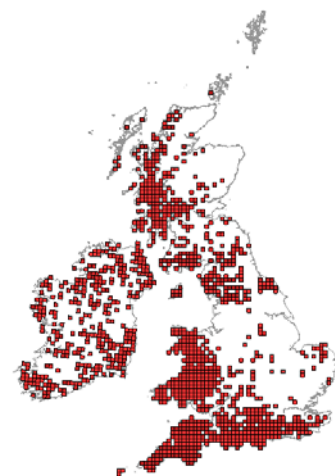
*Bryoria fuscescens*



*Candelaria reflexa*



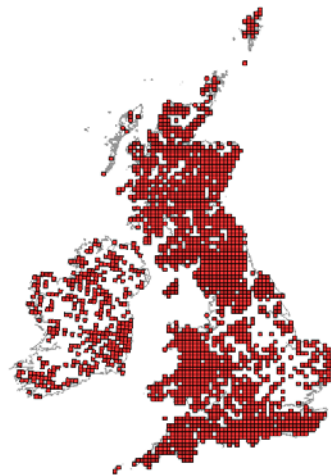
*Evernia prunstrati*



*Graphis elegans*



*Hypogymnia physodes*



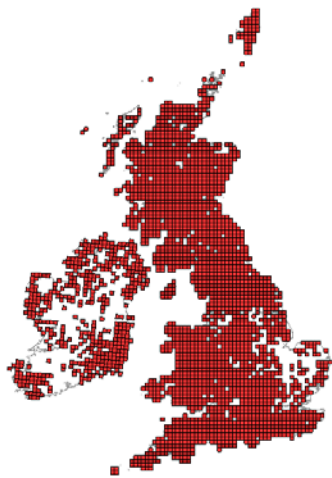
*Hypogymnia tubulosa*



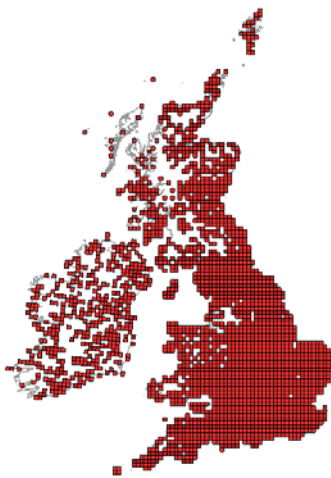
*Lecidella elaeochroma*



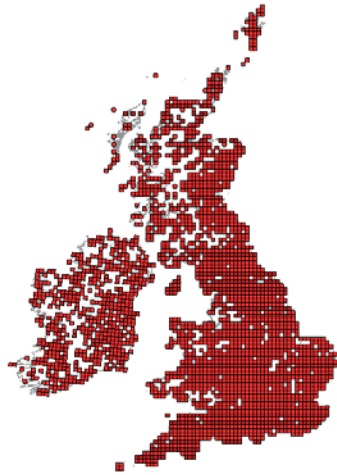
*Ochrolechia androgyna*



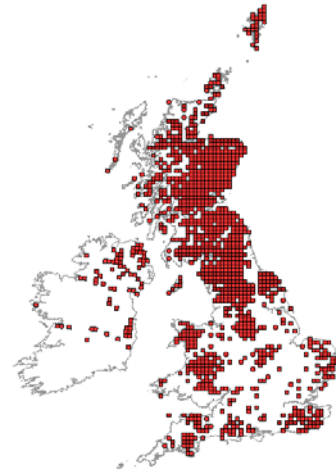
*Parmelia saxatilis*



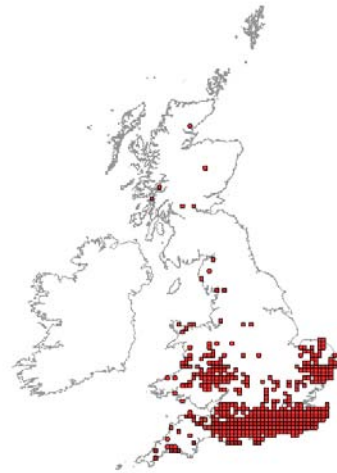
*Physcia adscendens*



*Physcia tenella*



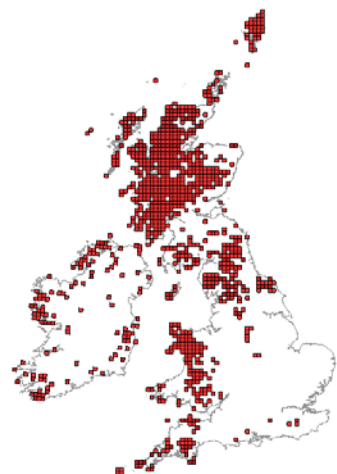
*Pseudevernia furfuracea* s.l.



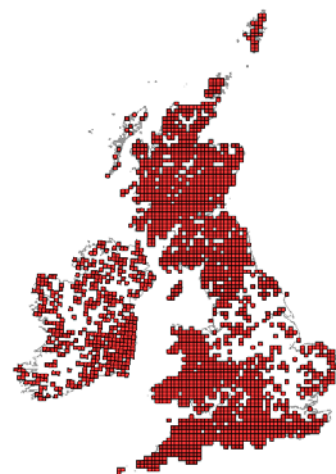
*Punctelia subrudecta* s.l.



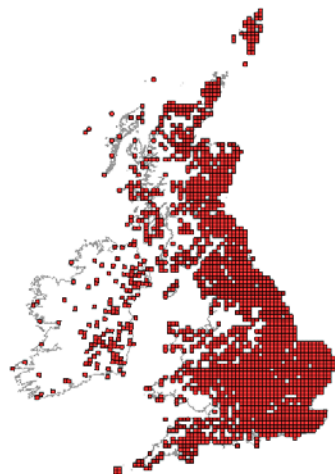
*Punctelia subrudecta* s.s.



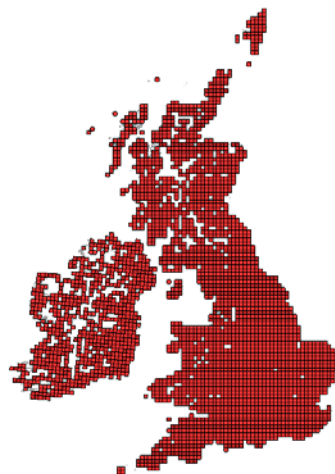
*Sphaerophorus globosus*



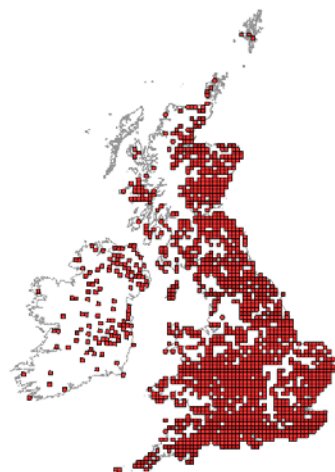
*Usnea subfloridana*



*Xanthoria candelaria* s.l.



*Xanthoria parietina*



*Xanthoria polycarpa*

## Appendix I List of contributors to the NBN dataset Source: [NBN Gateway](#) (2011)

Biodiversity Information Service for Powys and Brecon Beacons National Park
BLS Mapping Scheme dataset, 1750-2009 10km Full
BLS Scottish Sites Lichen Database 1750-2009 100m Full
BRERC February 2011 100m 1km
Bristol Regional Environmental Records Centre
British Lichen Society
British Mycological Society
BTCV Scotland
BTCV wildlife counts recording workshops 100m Full
Cambridge Lichen group data held by CPERC. 100m Full
Cambridgeshire & Peterborough Environmental Records Centre
CCW Regional Data : Mid-Wales, excluding vascular plants & bryophytes 100m Full
CCW Regional Data : South East Wales Non-sensitive Species Records 100m 10km
CCW Regional Data: all taxa (excluding sensitive species), West Wales 100m Full
CCW Regional Data: North Wales 100m Full
Centre for Environmental Data and Recording
Cofnod (North Wales Environmental Information Service)
Commissioned surveys and staff surveys and reports for SWT reserves. 100m Full
Countryside Council for Wales
Cumbria Biodiversity Data Centre
Cumbria Biodiversity Data Centre. Fungi and Lichen Observation Records. Pre-2010 for Cumbria. 100m 2km
DASSH Data Archive Centre Academic surveys 100m Full
DASSH Data Archive Centre expert sighting records 100m Full
Dorset Environmental Records Centre
Dorset Sites of Nature Conservation Interest (SNCI) species records 2000-2008 100m 1km
Dorset Sites of Nature Conservation Interest (SNCI) species records pre 2000 100m 1km
Dorset SSSI Species Records 1952 - 2004 (Natural England) 100m Full
Dr Francis Rose Field Notebook Project
EcoRecord
EHS Species Datasets 100m Full
Environmental Records Information Centre North East
ERIC North East non-sensitive species records 100m 1km
Field Notebook Records of Dr Francis Rose 1950's to 1990's 100m Full
Fungal Records Database of Britain and Ireland 100m 10km
Fungi records from Northern Ireland 100m 10km
GiGL professional survey records (Historic) 100m 1km
GiGL professional survey records (Last 10 years) 100m 1km
Greenspace Information for Greater London
Hampshire Biodiversity Information Centre
Hatfield Forest species data held by The National Trust. 100m Full
HBIC and partners species records 100m 1km
HBRG Fungus, Lichen and Lower Plants Dataset 100m Full
Hertfordshire Biological Records Centre
Highland Biological Recording Group
Humber Environmental Data Centre
Humber Environmental Data Centre - Non Sensitive Records from all taxonomic groups 100m 10km
Ickworth species data held by The National Trust. 100m Full
John Muir Trust
Joint Nature Conservation Committee
Kent & Medway Biological Records Centre
Leicestershire and Rutland Environmental Records Centre
Leicestershire and Rutland Fungi and Lichen Records 100m Full
Lichens & Lichenicolous Fungi: Records for Kent 100m Full
Littoral Marine data from Countryside Council for Wales (CCW) Technical Support (Research & Monitoring) Contracts, Wales 100m Full
Local Wildlife Site Surveys Berkshire 100m Full
Local Wildlife Site Surveys Oxfordshire 100m Full
Marine Biological Association
Marine Conservation Society
Marine flora and fauna records from the North-east Atlantic 100m Full
Marine Nature Conservation Review (MNCR) and associated benthic marine data held and managed by English Nature 100m Full
Marine Nature Conservation Review (MNCR) and associated benthic marine data held and managed by JNCC 100m Full
Marine Nature Conservation Review (MNCR) and associated benthic marine data held and managed by Scottish Natural Heritage 100m Full
Merseyside BioBank
National Trust



National Trust for Scotland (staff)
Natural England
Natural England species data for SSSI within Worcestershire from date of notification to present 100m 10km
NE Scotland fungus and lichen records 1800-2010 100m 1km
NE Scotland NTS properties species records 100m Full
NI Lichen Data set 100m 10km
Norfolk Biodiversity Information Service
Norfolk Fungi and Lichen records to March 2010 100m 10km
North & East Yorkshire Ecological Data Centre
North and East Yorkshire Ecological Data Centre - Non-sensitive Records from all taxonomic groups. 100m 10km
North Ayrshire Countryside Ranger Service
North East Scotland Biological Records Centre
North Merseyside General Recordsets 100m 2km
North Merseyside Marine Recordsets 100m 2km
Northern Ireland Environment Agency
Northern Ireland Fungus Group
Patrick Roper's Notebooks 100m 1km
Phase 2 Lowland Grassland Survey of Wales 100m Full
Plants, Bryophytes and Lichens recorded on Quinag in 2006-2007 100m Full
Porcupine Marine Natural History Society
RHS monitoring of native and naturalised plants and animals at its gardens and surrounding areas 100m Full
Rotherham Biological Records Centre
Rotherham Biological Records Centre - Non-sensitive Records from all taxonomic groups 100m 10km
Royal Horticultural Society
Scottish Borders Biological Records Centre
Scottish Natural Heritage
Scottish Wildlife Trust
Seasearch Marine Surveys 100m Full
SER Site-based Surveys 100m 10km
SER Species-based Surveys 100m 10km
Sheffield Biological Records Centre
Sheffield Biological Records Centre- Non-sensitive Records from all taxonomic groups. 100m 10km
Shropshire Ecological Data Network
Shropshire Ecological Data Network Database 100m Full
South East Wales Biodiversity Records Centre
Species data for Special Wildlife Sites within Worcestershire from date of notification to present. 100m 10km
Species within North Ayrshire from 1984 - Present 100m Full
Staffordshire Ecological Record
Staffordshire Wildlife Trust Nature Reserves Inventory 100m 10km
Stoke-on-Trent Environmental Survey results (1982-1984) 100m 10km
Suffolk Biological Records Centre
Suffolk Biological Records Centre (SBRC) dataset 100m 10km
Surrey Biodiversity Information Centre
Surrey Wildlife Trust Nature Reserves - Tranche 1 Species Records 100m 2km
Sussex Biodiversity Record Centre
SWT Scottish Borders Local Wildlife Site Survey data 1996-2000 - species information 100m Full
Thames Valley Environmental Records Centre
Tullie House Museum
Tullie House Museum, Cumbria Wildlife Trust survey records from 1970 - 2007 of Cumbria Wildlife Sites. Various. 100m Full
Vegetation surveys of coastal shingle in Great Britain 100m Full
Volunteer survey Data collated by DASSH, The marine Biodiversity Data Archive Centre 100m Full
WBRC Species data for Worcestershire collated by date. 100m 10km
WBRC Species data for Worcestershire collated by species group 100m 2km
Welsh Invertebrate Database (WID) 100m Full
West Wales Biodiversity Information Centre
Wicken Fen nature reserve species data held by The National Trust 100m Full
Wildlife Site Surveys, Hertfordshire 100m Full
Wildlife Trust for Birmingham and the Black Country Surveys 100m 10km
Wiltshire & Swindon Incidental Species Records 100m 1km
Wiltshire & Swindon Site-based Survey Records 100m 1km
Wiltshire and Swindon Biological Records Centre
Worcestershire Biological Records Centre
Worcestershire Wildlife Trust species data for owned and managed Reserves within Worcestershire from date of first acquisition to present. 100m 10km
Yorkshire Wildlife Trust
Yorkshire Wildlife Trust - Non-sensitive records from all taxonomic groups 100m 10km

**Appendix J** Short list of indicator species and the key characteristics that differentiate them from superficially similar species.

<i>Indicator Species</i>	<i>Characteristics</i>	<i>Similar Species</i>	<i>Differentiating Characteristics</i>	<i>Notes</i>
<i>Amandinea punctata</i>	Thallus smooth, C-, K-, UV+; soredia absent. Apothecia black round ca. 0.5mm diameter. Conidia oblong.	<i>Buellia disciformis</i>	Thallus K+ yellow.	<i>B. disciformis</i> mainly found in the Highlands and W. Scotland, W. Wales and S.W. England.
		<i>Buellia pulverea</i>	Thallus powdery, sorediate, C+ pink.	<i>B. pulverea</i> restricted to N. and W. British Isles.
		<i>Buellia schaeereri</i>	Conidia shortly-oblong to ellipsoid.	<i>B. schaeereri</i> is mainly found in upland regions of Scotland and Wales.
		<i>Lecidella elaeochroma</i>	Thallus smooth or slightly granular, K+ yellow, C+ orange. Apothecia ca. 1mm diam.	<i>L. elaeochroma</i> widely distributed in similar habitats to <i>A. punctata</i> .
<i>Arthonia radiata</i>	Thallus white to pale grey. Apothecia irregular to substellate, < 2 mm long without raised margins.	<i>Graphis scripta</i>	Apothecia straight to curved lirellae with raised lip-like margins.	Both <i>A. radiata</i> and <i>G. scripta</i> widespread throughout the British Isles.
		<i>Phaeographis dendritica</i>	Apothecia dendritically branched lirellae > 2 mm long without raised margins.	Mainly on smooth bark in nutrient poor sites in W. Wales and S. and S.W. England.
<i>Bryoria fuscescens</i>	Thallus long, pendulous and hair-like, brown to grey.	<i>Bryoria subcana</i>	N/A. All Bryoria spp. may be classified as nitrophobic with respect to [NH <sub>3</sub> ] on the basis that they are calcifuges (Smith <i>et. al.</i> , 2010)	<i>B. subcana</i> restricted to N. and W. Britain.

<i>Indicator Species</i>	<b>Characteristics</b>	<b>Similar Species</b>	<b>Differentiating Characteristics</b>	<b>Notes</b>
<i>Candelariella reflexa</i>	Thallus squamulose becoming sorediate, K-. Apothecia rare; ascus 8-spored.	<i>Caloplaca flavocitrina</i>	thallus granular sorediate K+ purple	common on base-rich bark.
		<i>Candelariella xanthostigma</i>	Thallus granular forming continuous yellow-green crust K-	Both <i>C. reflexa</i> and <i>C. xanthostigma</i> widespread throughout Britain on vertical trunks of rough-barked trees.
<i>Evernia prunastri</i>	Thallus sorediate, dorsiventral, K+ yellow; upper thallus greenish; lower thallus pale green to white.	<i>Pseudevernia furfuracea</i>	Thallus isidiate; upper thallus grey to grey-white; lower thallus black, sometimes white at lobe ends.	Both <i>E. prunastri</i> and <i>P. furfuracea</i> widespread throughout UK. <i>P. furfuracea</i> more common in upland and northern regions.
		<i>Ramalina</i> spp.	Thallus not dorsiventral, K-, greenish on both surfaces.	Both <i>E. prunastri</i> and <i>Ramalina</i> spp. widespread throughout UK.
<i>Graphis elegans</i>	Thallus pale grey with yellow-orange tinge, K+ red. Apothecia black, simple or branched lirellae with raised, lip-like margins.	<i>Graphis alboscripta</i>	Thallus K+ pale yellow. Apothecia pale.	<i>G. alboscripta</i> restricted to western coast of Scotland.
		<i>Graphis scripta</i>	Thallus K-. Apothecia black, straight to curved.	Both <i>G. elegans</i> and <i>G. scripta</i> widespread throughout the British Isles.
<i>Hypogymnia physodes</i>	Thallus lobes appressed, flattened, without perforations; soredia arising from the inner surface of split upturned lobe ends.	<i>Hypogymnia tubulosa</i>	Thallus lobes erect, tubular and finger-like; soredia arising on the lobe ends.	Both <i>H. physodes</i> and <i>H. tubulosa</i> widespread. <i>H. tubulosa</i> typically more common on twigs and branches than trunks.
		<i>Menegazzia terebrata</i>	Thallus perforated.	<i>M. terebrata</i> restricted to high rainfall areas on the western edge of Britain.

<i>Indicator Species</i>	<b>Characteristics</b>	<b>Similar Species</b>	<b>Differentiating Characteristics</b>	<b>Notes</b>
<i>Hypogymnia tubulosa</i>	Thallus lobe tips erect, tubular, without perforations; soredia arising on the lobe ends.	<i>Hypogymnia physodes</i>	See <i>H. physodes</i> .	See <i>H. physodes</i> .
		<i>Menegazzia terebrata</i>	See <i>H. physodes</i> .	See <i>H. physodes</i> .
<i>Lecidella elaeochroma</i>	Thallus smooth or slightly granular, C+ orange, K+ yellow. Apothecia black, to 1mm diameter.	<i>Buellia disciformis</i>	Thallus smooth, C-.	<i>B. disciformis</i> found in the N. and W. Of Britain.
		<i>Fuscidea lightfootii</i>	Thallus warted or distinctly granulose, C-.	<i>L. elaeochroma</i> and <i>F. lightfootii</i> widespread. <i>F. lightfootii</i> less common in C. and E. England.
		<i>Megalaria grossa</i>	Thallus smooth to slightly verrucose, C-, K-.	<i>M. grossa</i> found in the N. and W. of Britain.
<i>Ochrolechia androgyna</i>	Thallus white to dark grey, C+ scarlet red; isidia absent; soralia rounded, soredia yellow-green.	<i>Ochrolechia microstictoides</i>	Thallus C+ yellow; Soralia pale grey-white.	<i>O. microstictoides</i> more common in the N. Britain and upland regions.
		<i>Ochrolechia tartarea</i>	Thallus C+ orange-red; soralia absent.	<i>O. tartarea</i> frequent in oceanic montane areas.
		<i>Pertusaria coccodes</i>	Thallus C-; soralia absent; isidiate.	<i>P. coccodes</i> restricted to S. and E. of Britain.

<i>Indicator Species</i>	<i>Characteristics</i>	<i>Similar Species</i>	<i>Differentiating Characteristics</i>	<i>Notes</i>
<i>Ochrolechia androgyna</i>	Thallus white to dark grey, C+ scarlet red; isidia absent; soralia rounded, soredia yellow-green.	<i>Pertusaria flavida</i>	Thallus yellowish, C+ orange; soralia absent; isidiate.	<i>P. flavida</i> restricted to E. Britain.
		<i>Pertusaria hemisphaerica</i>	Thallus grey, soralia white hemispherical, C+ carmine red.	Both <i>O. androgyna</i> and <i>P. hemisphaerica</i> widespread throughout the British Isles.
		<i>Pertusaria multipuncta</i>	Thallus grey, with white soralia, thallus and soredia C-.	<i>P. multipuncta</i> recorded mainly in S. and S.W. England, Wales and W. Scotland.
<i>Parmelia saxatilis</i>	Thallus with white pseudocyphellae arising from margins; isidiate; soredia absent.	<i>Parmelia sulcata</i>	Thallus with pseudocyphellae that become sorediate.	Both <i>P. saxatilis</i> and <i>P. sulcata</i> widely distributed throughout the UK.
<i>Physcia adscendens</i> and <i>P. tenella</i>	Thallus K+ yellow; soredia on lobe tips; cilia pale, sometimes dark-tipped. Apothecia infrequent, sparse when present.	<i>Physcia leptalea</i>	Soredia absent. Apothecia abundant.	Both <i>P. adscendens/tenella</i> widespread throughout Britain. <i>P. leptalea</i> more commonly found in S. and W. Britain.
<i>Pseudevernia furfuracea</i>	Thallus isidiate; upper surface grey to grey-white; lower surface black, sometimes white at lobe ends.	<i>Evernia prunastri</i>	See <i>Evernia prunastri</i> .	See <i>Evernia prunastri</i> .
<i>Punctelia subrudecta</i>	Thallus lower surface light brown to brown, lobe edges not pruinose. Soralia punctiform, arising from pseudocyphellae on the upper surface, C+ red.	<i>Punctelia borreri</i>	Thallus lower surface black. Soralia C+ pink	<i>P. borreri</i> recorded predominantly in S. England.
		<i>Punctelia jeckeri</i>	Thallus lower surface light brown to brown, lobe edges pruinose. Soralia mainly on the margins of the thallus, C+ red.	<i>P. jeckeri</i> widespread, but not found in N. and C. Scotland.
		<i>Punctelia reddenda</i>	Thallus lower surface black. Soralia C-.	<i>P. reddenda</i> recorded predominantly in S. England and Wales.

<i>Indicator Species</i>	<b>Characteristics</b>	<b>Similar Species</b>	<b>Differentiating Characteristics</b>	<b>Notes</b>
<i>Sphaerophorus globosus</i>	Thallus branches brittle, rounded and dichotomously branched.	<i>Bundophoron melanocarpum</i>	Thallus branches flattened and palmately branched.	<i>B. melanocarpum</i> restricted to N.W. Scotland and Wales.
<i>Usnea</i> spp.	Thallus shrubby or pendant, yellow-green to grey-green. Core solid in cross-section.	<i>Alectoria sarmentosa</i>	Thallus pendulous hair-like, dichotomously branched. Core hollow in cross-section.	<i>A. sarmentosa</i> restricted to the Scottish Highlands.
<i>Xanthoria candelaria</i> s.l.	Thallus • 2 cm, yellow-orange, K+ purple, marginally sorediate; rhizines absent.	<i>Xanthoria ucrainica</i>	N/A. <i>X. candelaria</i> s.l. groups together <i>X. candelaria</i> and <i>X. ucrainica</i> .	Both <i>X. candelaria</i> and <i>X. ucrainica</i> widespread throughout Britain.
<i>Xanthoria parietina</i>	Thallus • 15 cm, K+ purple; upper surface yellow-orange to greenish in shade; lower surface white; rhizines present. Apothecia abundant.	<i>Xanthoria polycarpa</i>	See <i>X. polycarpa</i> .	See <i>X. polycarpa</i> .
<i>Xanthoria polycarpa</i>	Thallus • 3 cm, K+ purple; upper surface orange-yellow; lower surface white; rhizines present. Apothecia abundant on ± erect lobes.	Juvenile <i>Xanthoria parietina</i>	Lobes large appressed not erect. Apothecia not on erect stalk-like lobes.	Both <i>X. parietina</i> and <i>X. polycarpa</i> widespread throughout Britain.

## Appendix K – Examples of the four FL<sub>AN</sub> scoring systems utilised

	Tree 1 ( $n = 1$ )				Tree 2 ( $n = 2$ )				Tree 3 ( $n = 3$ )				Tree 4 ( $n = 4$ )				Tree 5 ( $n = 5$ )							
	East		South		West		East		South		West		East		South		West		East		South		West	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep	Hp	Ps	Ep	Hp	Ep	Hp	Ps	
	Ep	Hp	Ep	Hp	Pf	Ep	Hp	Ep	Hp	Ep	Hp	Bf	Ep	Hp	Ps	Ep								

$$\text{NUFL}_{\text{AN}} = \frac{\bullet \cdot (\text{A}-\text{N})}{n} = \frac{3+3+3+3+2}{5} = 2.8$$

$$NFFL_{AN} = \frac{\bullet (Aa_i - Nn_j)}{n} = \frac{12+18+12+15+6}{5} = 12.6$$

$$AUFL_{AN} = \frac{\bullet (A_i - N_i)}{n} = \frac{9+11+8+8+5}{5} = 8.2$$

$$\text{AFFL}_{\text{AN}} = \frac{\bullet (A_{j,q} - N_{j,n})}{n} = \frac{36+66+32+40+15}{5} = 37.8$$

### Nitrophobes

*Bryoria fuscescens* (Bf)  
*Parmelia saxatilis* (Ps)  
*Ochrolechia androgyna* (Oa)  
*Usnea* spp. (Us)  
*Hypogymnia physodes* (Hp)  
*Hypogymnia tubulosa* (Ht)  
*Evernia prunastri* (Ep)  
*Pseudevernia furfuracea* (Pf)  
*Sphaerophorus globosus* (Sg)

## Nitrophiles

*Xanthoria parietina* (Xa)  
*Xanthoria polycarpa* (Xo)  
*Xanthoria candelaria* s.l. (Xc)  
*Physcia adscendens/tenella* (Pa)  
*Amandinea punctata* (Ap)  
*Candelariella reflexa* (Cr)  
*Lecidella elaeochroma* (Le)  
*Arthonia radiata* (Ar)  
*Punctelia subrudecta* (Pu)

**Notes:**

Quadrats on each aspect clumped together and treated as one quadrat in example, as in assessment.

